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CLA-Rich Soy Oil Shortening, Chocolate Paste, and Chocolate Bar Production and Characterization

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CLA-Rich Soy Oil Shortening, Chocolate Paste, and Chocolate Bar Production and Characterization

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By

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ABSTRACT

Conjugated linoleic acid-rich soy oil (CLARSO) has been shown to have numerous health benefits, including anti-obesity and anti-carcinogenic properties. This oil was previously used to produce CLA-rich margarine that provided the recommended daily value of CLA and showed physical characteristics similar to commercially available margarine. The objectives of this study were to produce CLA-rich shortenings and analyze their physical properties relative to commercially available shortenings and soy oil control shortenings and to produce CLA-rich chocolate bars and pastes by replacing a portion of the fat with CLARSO and compare the physical properties of these pastes/bars to controls made with either soy oil or traditional fats. The shortenings were prepared and their rheology, thermal behavior, solid fat content, and microstructure were determined and compared to the commercial samples. CLARSO, soy oil, and traditional fat blends were used to prepare bars/pastes. Rheology, firmness, and thermal behavior of the pastes and fracturability, hardness, and thermal behavior of the bars were determined relative to control samples. The CLA-rich shortening samples showed similar rheological properties to the commercial samples and exhibited more solid-like behavior than the soy oil control samples. The CLA-rich shortenings had higher solid fat content (% SFC) than the soy oil controls. CLA-shortenings released more heat upon crystallization and absorbed more heat upon melting than did the soy oil shortenings, indicating a comparatively higher crystalline fraction. However, the oil used did not affect the shortening crystallization temperatures and there was no observable difference in microstructure of the CLA and soy oil shortenings, likely due to palm stearin having a greater effect on the crystal structure than did the oil. The Crisco

commercial shortening had a more uniform crystal structure than did the experimental shortenings, likely due to the Crisco standardized industrial process. The CLARSO chocolate pastes/bars contained no additional saturated fat relative to soy oil controls but the pastes had more solid rheology and were firmer and the bars had a higher fracture force relative to soy oil controls. Relative to non-soy controls, CLARSO pastes had similar rheology and CLARSO bars had similar fracturability, despite containing less saturated fat. The fat crystals of all samples were in the same polymorphic form. Therefore, CLARSO has the ability to produce chocolate pastes/bars with similar physical properties as traditional products containing more saturated fat.

Key words

Conjugated linoleic acid-rich soy oil (CLARSO), shortening, rheology, differential scanning calorimetry (DSC), solid fat content (SFC), chocolate paste, chocolate bars, texture

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1. Mayfield S., Shinn S.E., Proctor A., Dewettinck K., Patel A. (2015). CLA-rich soy oil shortening production and characterization. *J. Am. Oil Chem. Soc.* (in review).
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INTRODUCTION

Conjugated linoleic acid (CLA) is an 18-carbon dietary fatty acid, consisting of various positional and geometric isomers, found mostly in dairy and bovine foods as a product of rumen fermentation (1). CLA has been shown to have many possible positive effects on human health, including anti-carcinogenic properties (2, 3), the ability to combat obesity and atherosclerosis (4, 5), lower the risk of diabetes (6), and improve immune function (7).

Approximately 3.2 grams of CLA need to be consumed daily to realize the health benefits. However, the animal CLA sources are also high in saturated fat and cholesterol (8, 9). Therefore, a rich source of CLA that is low in saturated fats and cholesterol would be desirable. Soy oil contains 50% linoleic acid (LA) which has the potential to be isomerized to CLA and could be used to produce CLA-rich food products (10). Jain and Proctor (11) developed a process to produce 20% CLA-rich soy oil (CLARSO) by photoisomerization of soy oil LA in the presence of an iodine catalyst. Gilbert *et.al.* (7) demonstrated that when obese Zucker rats were fed this CLA-rich oil, their total serum cholesterol and LDL cholesterol were reduced by 50% relative to rats fed a soy oil control diet and their liver weight was reduced by 35%. This CLA-rich oil also increased the expression of the PPAR-γ gene, a gene that alleviates insulin resistance and decreases cardiovascular risk factors (7). However, the necessity to remove iodine made the process difficult to commercialize.

A heterogeneous catalysis process was developed by Shah and Proctor (12) that produces 20% CLARSO in 2 hours without the use of solvents by adapting the oil deodorization process, which is used routinely in industry. A ruthenium-on-carbon catalyst is combined with the oil in a high temperature, vacuum distillation technique as used in the deodorization step in general oil processing. The catalyst can be centrifuged and filtered out of the oil in a relatively simple process, rendering the oil safe for human consumption (13).

Ruan and Proctor (14) found that CLARSO had a higher melting temperature and viscosity than RBD soy oil. These differences were attributed to greater intermolecular hydrophobic interaction forces of the CLA conjugated double bonds *trans-trans* fatty acids of CLARSO. A CLA-rich margarine was subsequently developed by Shah *et. al.* (15) and the firmness, rheology, thermal behavior, solid fat content (SFC) and microstructure of CLARSO margarine were compared to a soy oil control margarine and a commercially available margarine. CLA-rich margarine was firmer, better able to withstand deformation by stress, and had a higher solid fat content than the soy oil margarine. It also had comparable physical properties to commercial margarine. A standard 7 g serving of this CLA-rich margarine provided 0.6 g of CLA, and therefore 5 servings would provide the recommended 3.2 g of CLA per day while providing only 185 Calories. The results of this study illustrate the effectiveness of CLARSO as a replacement for more saturated fats in food products and thus the practicality of developing more foods with this oil.

Shortening, like margarine, is a food ingredient commonly used in baked goods such as biscuits and pie crusts, foods readily consumed by those at risk of obesity and cardiovascular disease. It has a semisolid structure composed of a crystalline fat network that determines it microstructure and therefore its physical properties (16), including rheological, mechanical, and thermal behavior (17). Therefore, the oils and fats used to produce shortenings will have a significant effect on product quality. Chocolate is a fat-based food whose physical properties are also dependent upon its polymorphic crystal structure. A recent European Parliament and Council directive mandated that no more than 5% of the cocoa butter in chocolate bars be replaced with an alternative fat to maintain the EU standard of identity (Directive 2000/36/EC, European Parliament and Council, June 23, 2000). It will be of interest to know how such a replacement of cocoa butter with CLARSO, and the replacement of palm oil with CLARSO in chocolate paste, will affect chocolate products.

OBJECTIVES

The objectives of this study were to:

1) Produce CLA-rich shortenings and determine their physical functional properties relative to those obtained using a soy oil control and commercial shortenings.

2) Determine the functional physical properties of chocolate paste prepared by replacing 25% of a palm oil/canola oil mixture with CLARSO, relative to control pastes obtained by replacing 25% of the palm/canola mixture with soy oil, and a control made solely with the palm/canola oil mixture.

3) Determine the functional physical properties of chocolate bars prepared by replacing 5% of the cocoa butter with CLARSO relative to control bars obtained by replacing 5% of the cocoa butter with soy oil, and a control made solely with cocoa butter.

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CHAPTER 1

CLA-rich shortening production and characterization.

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EXPERIMENTAL PROCEDURES

Materials

Refined, bleached, and deodorized (RBD) soy oil was obtained from Riceland Foods (Stuttgart, AR, USA). Palm stearin (referred henceforth as stearin) and shortening made with partially hydrogenated vegetable oil were provided by Archer Daniels Midland (ADM) (Decatur, IL, USA). Commercial *Crisco* shortening was purchased from a Walmart store (Bentonville, AR, USA). All chemicals used were reagent grade.

Methods

CLA-Rich Soy Oil Production and Fatty Acid Analysis: The method of Shah and Proctor (12) was used to produce CLA-rich soy oil (CLARSO) through heterogeneous catalysis of refined bleached deodorized (RBD) soy oil. The fatty acid profile of the RBD and

CLARSO oil was determined in triplicate by the method of Lall *et. al.* (18). Oil fatty acids were converted to FAMES and then subjected to GC-FID analysis in triplicates.

Shortening Preparation: CLA-rich shortenings were prepared by methods provided by Archer Daniels Midland (Bryan Kickle, Archer Daniel Midland, personal communication, October 1, 2013). Shortenings with a range of 60-80% CLARSO, 40-20% stearin with a total weight of 400g were prepared as shown in Table 1.1. The shortening formulations were each heated in a 500-mL glass beaker with constant stirring to a temperature of approximately 70-80°C, or until fully melted. The fat blends were each poured into a stainless-steel 500-mL beaker, placed in a 0°C water bath, and cooled to 10°C. During cooling, the blend was constantly stirred with an electric hand mixer (Rival 125 Watt Hand Mixer, Sunbeam Products, Inc., Boca Raton, FL, USA) set on the lowest speed to ensure uniform crystal formation. The shortening samples were placed in sealed 50-mL plastic centrifuge tubes and stored at 20°C prior to analysis. Control shortenings with the same oil: stearin ratio were prepared with conventional soy oil from the same batch used to produce CLA-rich oil. Additional control shortenings used were a 1) partially hydrogenated vegetable oil (PHO) (ADM, Chicago, IL) and 2) a commercial *Crisco* shortening.

Rheology Determination: The rheology of all samples was determined in duplicate using an AR 2000 Rheometer (TA instruments, New Castle, DE, USA) with a crossed-hatched, parallel plate geometry (diameter = 40 mm). The geometry gap for analysis was set at 1000 µm. Oscillatory shear tests (strain and frequency sweeps) were preformed to analyze the gel strength and viscoelastic behavior of the samples. The strain sweep

consisted of increasing the strain from 0.0001 to 100 while holding the frequency (*v*) constant at 1 Hz. The frequency sweep consisted of subjecting the sample to a frequency that was increased from 0.1 to 100 Hz while holding the strain constant at 0.04. Both strain and frequency sweeps were performed at a constant temperature of 20°C (which was also the storage temperature). For the strain sweeps, the elastic modulus G' was measured as a function of strain and used to determine the resistance of the sample to deformation as the stress on the sample was increased. For the frequency sweeps, G' was measured as a function of angular frequency ($\omega = 2\pi v$) and used to determine at what particular frequency the structure of the sample began to break down.

Solid Fat Content (SFC) Determination: The solid fat content of the samples was measured as a function of temperature in triplicate analysis using a 23 MHz ^{1}H NMR Maran instrument (Oxford Instruments, Oxfordshire, UK). Prior to analysis, the samples were melted in a 60°C oven, placed in a 0°C water bath for one hour, and placed in a 5°C water bath for 30 minutes. Measurements were taken in 5°C increments over a range of 5-55°C. The samples were allowed to equilibrate at each temperature for 30 minutes prior to taking measurements.

Thermal Behavior: Thermal analysis was determined by differential scanning calorimetry (DSC). Experiments were performed in triplicate analysis using a Q1000 Tzero DSC (TA Instruments, New Castle, DE, USA). Indium (enthalpy and temperature), azobenzene (temperature), and undecane (temperature) were used to calibrate the instrument prior to analysis. An empty pan was used as a reference and the instrument was purged with nitrogen. Five to ten mg of each sample was placed in

hermetic aluminum pans and the pans were sealed. The crystallization behavior of the samples was determined by equilibrating the sample at 80°C, keeping it isothermal for 10 minutes, and decreasing the temperature 5°C per minute to a temperature of -80°C. The isothermal crystallization behavior was then determined by keeping the sample isothermal for 10 minutes, increasing the temperature 10°C per minute to 20°C, and keeping the sample isothermal for 180 minutes. The melting behavior was determined by increasing the temperature 5°C per minute to 80°C.

Non-isothermal crystallization, isothermal crystallization, and post-crystallization melting behaviors were determined. The resulting DSC profile curves were integrated using the Universal Analysis software (TA Instruments, New Castle, DE, USA).

Microstructure: A Leica DM2500 microscope (Leica Microsystems CMS GmbH, Wetzlar, Germany) was used to observe the microstructure of the samples. Slides were prepared by placing a small droplet of shortening and then placing a coverslip on top to flatten and spread the droplet. The slides were placed under transmitted polarized light at 20°C to visualize the microstructure.

Data Analysis: All statistical analyses were performed using JMP 10 (SAS Institute, Inc., Cary, NC) statistical software. The strain and frequency sweeps, SFC, and DSC results were analyzed by comparing the overall means in a one-way ANOVA using Tukey's HSD test with an α-level of 0.05. The G' values for the strain and frequency sweeps were transformed logarithmically to obtain a better comparison.

RESULTS AND DISCUSSION

CLA-Rich Oil Fatty Acid Analysis

Table 1.2 shows the fatty acid data from FAMEs analysis of CLARSO and soy oil used to produce shortenings. The soy oil had a typical profile with 54.74% linoleic as the predominant fatty acid. There was no significant difference between the total saturated fat content of CLARSO and soy oil. The CLARSO contained 15.76% *cis,trans/trans,cis* CLA and 5.07% *trans,trans* CLA, with a total CLA content of 20.83%. Therefore, 15.4 g of this oil would need to be consumed daily in order to receive the recommended 3.2 g to reap the health benefits.

Shortening Characterization

Rheology

Figure 1.1 shows elastic modulus (G') as a function of percent strain for all shortening samples. There were no clearly defined linear viscoelastic regions or critical deformation values for any of the samples. All samples showed a slow, steady decrease in the G' as the strain was increased. The rheology curves of all samples had similar shapes. The mean G' values for one decade of strain, from 0.001 to 0.01, were compared for each sample to determine any statistically significant differences between samples, as shown in Table 1.3. As the amount of oil in the samples was increased, the mean G' value decreased. The 60% soy oil sample had a significantly higher mean G' value than did the 60% CLARSO sample. The 70% CLARSO and soy oil samples were not significantly different. The CLARSO sample had a significantly higher G' value than its paired soy oil sample at oil levels of 65%, 75%, and 80%. These results indicated that there was no underlying trend between the type of soy oil used and the

solid-like behavior of the sample. It was proposed that the solid stearin played a greater role in the structure of the samples than did the liquid oil. The Crisco sample had a mean G' value that fell between that of 65% soy oil and 70% CLARSO/soy oil. The PHO sample had a mean G' value that was not significantly different than that of the 65% CLARSO sample. Based on these results, it was determined that CLARSO shortenings could replace commercially available shortenings without compromising the flow behavior.

Figure 1.2 shows the plots of G' as a function of angular frequency. The strain was held constant at 0.004 throughout the experiment, because the samples had not significantly deformed at this strain, as seen from the strain sweep in Figure 1.1. The G' was independent of frequency at this strain value, indicating that the samples behaved as solids below their critical strain values. The curves for all of the samples had similar linear shapes, and as the percent oil in the samples was increased, the value of G' decreased. This was consistent with the strain sweep results. The overall mean G' values were compared for each sample to determine any statistically significant differences between samples, as shown in Table 1.4. The CLARSO and soy oil samples with the same percentage of oil produced significantly different results at all oil levels except 70%. The CLARSO samples containing 65%, 75%, and 80% oil had significantly higher overall mean G' values than their paired soy oil samples. It was determined that, in general, CLARSO gave the samples more solid-like behavior than did soy oil. The Crisco sample had an overall mean G' value between that of the 65% soy oil sample and the 70% CLARSO/soy oil samples, and the PHO sample had an overall mean value that was not significantly different from that of the 60% soy oil. Therefore, the frequency sweep

showed similar results for the Crisco and PHO samples as did the strain sweep, again indicating that CLARSO shortenings could be an alternative to commercially available shortenings.

Thermal Behavior

Figure 1.3 shows the solid fat content as a function of temperature for all samples. All curves had similar shapes except for that of the PHO sample. This sample had a much higher solid fat content at lower temperatures (5-20°C) but declined more rapidly as the temperature was increased than did the other samples. The mean SFC values at 20°C were compared for each sample to identify any statistically significant differences, as shown in Table 1.5. By comparing the values at 20°C, rather than over the entire range, the SFC results could be equated to the rheology results, where all measurements were conducted at 20°C. There was no significant difference in SFC values between paired CLARSO and soy oil samples at oil levels of 60%, 65%, and 70%. However, the 75% and 80% CLARSO samples had significantly higher SFC values than did their paired soy oil samples. These results indicated that although there was no difference in SFC between CLARSO/soy oil samples with lesser amounts of oil (and greater amounts of stearin), as the amount of oil was increased, there was an observed difference. It was determined that stearin had a greater effect on the thermal behavior than did the liquid oil, especially in the samples that contained more stearin. These results were conclusive with those of the strain sweep, where it was concluded that stearin affected the physical behavior more so than did the liquid oil. The Crisco sample had an SFC value that was not significantly different from that of 70% CLARSO/soy oil and 75%

CLARSO. This was inconsistent with the rheology results, where Crisco displayed more solid-like behavior than 70% oil samples. It was proposed that the industrial process (involving a controlled temperature-time profile) used to produce Crisco created a harder product without adding additional solid fats. Shah *et. al.* (15) came to a similar conclusion when comparing CLA-rich margarine with commercially available margarine: the commercial margarine had similar rheological properties to CLA-rich margarine but had a lower solid fat content. Therefore, the textural characteristics of the samples were not influenced by SFC alone but also by the crystal types and network structures formed when the fats are crystallized (19). The PHO sample had a significantly higher SFC value than did any of the other samples, indicating that it contained the greatest amount of saturated fats.

Figure 1.4 shows the DSC spectrum for the crystallization cycle of 60% CLARSO. The temperature was steadily lowered and the heat flow was measured as a function of temperature. For all samples, there were two peaks observed during this cycle, and it was determined that the first peak, which appeared at a higher temperature, represented the crystallization of the solid/saturated fats and the second peak, which appeared at a lower temperature, represented the crystallization of the liquid/unsaturated fats (17). There were seven different parameters measured in this cycle: the onset of the first peak (°C), the maximum value of the first peak (°C), the onset of the second peak (°C), the maximum value of the second peak (°C), the enthalpy (integration) of the first peak (J/g), the enthalpy of the second peak (J/g), and the total enthalpy (J/g).

The samples with oil levels of 60%, 65%, and 75% showed no significant difference between the peak 1 onset points of the paired CLARSO and soy oil samples. The soy oil samples showed a significantly higher onset temperature than their paired CLARSO samples at oil levels of 70% and 80%. It was observed that as the percent oil in the samples was increased, the onset temperature of peak 1 decreased. These results indicated that samples with a higher amount of solid fat began to crystallize at a higher temperature. However, no relationship was observed between the type of oil used and the onset of crystallization. The Crisco sample had a significantly higher peak 1 onset temperature than of any of the samples and the PHO sample had an onset temperature that was not significantly different from that of the 60% soy oil sample.

There was no significant difference in the maximum peak 1 temperature observed between the paired CLARSO/soy oil samples for any of the oil levels. Therefore, it was determined that the type of oil used did not affect the mean solid fat crystallization temperature. As the percent oil in the samples was increased, the maximum peak 1 temperature decreased. This indicated that samples with less solid fat crystallized at a lower temperature which is expected because of a higher solubility of solid fat in these samples due to a proportionally higher liquid oil content. The Crisco had a significantly higher maximum peak 1 temperature than any of the other samples, and the PHO sample had a significantly higher maximum temperature than all of the samples except Crisco. This indicated that these samples crystallized at a higher temperature.

The 60%, 70%, and 75% oil samples showed no significant difference in peak 2 onset temperature between the paired CLARSO/soy oil samples. The CLARSO samples showed a significantly higher onset temperature than their paired soy oil samples at oil levels of 65% and 80%. As the percent of oil in the samples was increased, the peak 2 onset temperature decreased, indicating that samples with greater amounts of unsaturated fat began to crystallize at a lower temperature. However, there was no relationship observed between the type of oil used and the onset temperature. The Crisco sample had a significantly lower peak 2 onset temperature than any of the other samples and the PHO sample had a significantly higher onset temperature than any of the other samples.

There was no significant difference observed in maximum peak 2 temperature between the paired CLARSO/soy oil samples at oil levels of 60%, 70%, 75%, and 80%. The 65% CLARSO sample had a significantly higher maximum peak 2 temperature than the 65% soy oil sample. These results indicated that, in general, the type of oil used does not affect the temperature at which the oil crystallizes. There was no observable relationship between the amount of oil in the sample and the temperature at which this oil crystallized. The Crisco sample had a significantly lower maximum peak 2 temperature than any of the other samples and the PHO sample had a significantly higher maximum peak 2 temperature than any of the other samples.

All paired CLARSO/soy oil samples, except for the 75% oil samples, showed no significant difference in the enthalpy of the first peak. The 75% CLARSO sample had a significantly higher enthalpy than the 75% soy oil sample. It was determined that the type

of oil used did not affect the heat released when the solid fats in the sample crystallized but as the percent of oil in the samples was increased, the enthalpy of peak 1 decreased. The result is consistent with the SFC results where an increase in the oil percent resulted in lowering of SFC values irrespective of the type of oil used. The Crisco sample had an enthalpy that was not significantly different from that of the 60% soy oil sample and the PHO sample had an enthalpy that was not significantly different from that of the 70% CLARSO/soy oil and 75% CLARSO samples.

At all levels of oil, the CLARSO samples showed a significantly higher peak 2 enthalpy than their paired soy oil samples. However, there was no relationship observed between the amount of oil present in the samples and the peak 2 enthalpy. These results indicated that more heat was released per gram when CLARSO crystallized than when soy oil crystallized. This greater heat release showed that the CLARSO samples had a more thermodynamically stable, and therefore more solid-like, crystal matrix (20). This is consistent with the strain and frequency sweep and SFC results, where the CLARSO samples were shown to behave more like solid fats than the soy oil samples. The Crisco sample had a significantly lower peak 2 enthalpy than any of the other samples and the PHO sample had a significantly higher peak 2 enthalpy than any of the other samples.

At all oil levels except 60%, the CLARSO samples had a significantly higher total enthalpy than did their paired soy oil samples. This result was consistent with that of the peak 2 enthalpy: the CLARSO samples released more heat per gram than did the soy oil samples, therefore indicating that CLARSO samples had a comparatively higher

crystalline fraction. The Crisco sample had an enthalpy that was not significantly different from that of the 75% soy oil and 80% soy oil samples, and the PHO sample had a significantly higher total enthalpy than did any of the other samples.

Figure 5 shows the DSC spectrum for the isothermal crystallization cycle of the 60% CLARSO sample. The temperature was held constant and the heat flow was measured as a function of time. There was a single peak observed for each sample and there were four parameters measured: the peak onset (min), the peak maximum (min), the peak offset (min) and the enthalpy (J/g).

The 60%, 65%, and 70% oil samples showed no significant difference between the onset times of the paired CLARSO/soy oil samples. The CLARSO samples showed a significantly greater onset time than their paired soy oil samples at oil levels of 75% and 80%. No relationship was observed between the amount of oil and the onset time. The Crisco sample showed no significant difference in onset time from the 60 and 65% CLARSO and soy oil samples and the 80% soy oil sample. The PHO sample showed no significant difference from the 70% CLARSO and soy oil samples and the 75% soy oil samples. It was therefore determined that, overall, the type/amounts of fatty acids present did not influence the isothermal crystallization of the shortening sample, and that other factors, such as ambient temperature, container geometry, and any slight variations in sample preparation play a more significant role in this process (21).

At all levels of oil except 80%, the CLARSO/soy oil pairs showed no significant difference in peak isothermal crystallization time. The 80% CLARSO sample had a greater peak time than did the 80% soy oil sample. There was no relationship observed between the amount of oil present and the peak time. The Crisco sample had a peak time that was not significantly different from that of the 60%, 65%, 70% and 75% CLARSO/soy oil samples, the 80% soy oil sample, and the PHO sample. Therefore, it was again concluded that the type/amount of fatty acids present in the shortening samples did not contribute to isothermal crystallization time, and that other factors played a more important role.

At all levels of oil, the CLARSO samples had a significantly higher offset time than did their paired soy oil samples. However, there was no relationship observed between the amount of oil and the offset time. The Crisco sample had an offset time that was not significantly different from that of the 60% and 65% CLARSO/soy oil samples and the 70% soy oil sample. The PHO had an offset time that was not significantly different from any of the CLARSO samples. These results again indicated that the type/amount of fatty acids did not influence the crystallization time.

At all oil levels except 70%, there was no significant difference in enthalpy within the paired CLARSO/soy oil samples. As the percent oil in the samples was increased, the enthalpy was decreased. These results indicated that the type of oil used did not influence the heat released when the samples crystallized isothermally, and that samples with greater amounts of solid fats released more heat upon crystallization. The Crisco

sample had an enthalpy that was not significantly different from that of the 80% CLARSO/soy oil samples and the PHO sample had an enthalpy that was not significantly different from that of the 60% and 65% CLARSO/soy oil samples.

Figure 1.6 shows the DSC spectrum for the melting cycle of the 60% CLARSO sample. The temperature was steadily increased and the heat flow was measured as a function of temperature. There was a single peak observed and there were four parameters measured: the onset temperature, the maximum temperature, the offset temperature, and the enthalpy.

There was no significant difference in onset temperature between the CLARSO/soy oil pairs at any levels of oil. With the exception of the 70% CLARSO and 80% soy oil samples, there was no significant difference observed between the onset temperatures of any of the samples. The Crisco and PHO samples had onset temperatures that were not significantly different from those of any of the other samples. These results indicated that all samples began melting at the same temperature, which also suggests that the fat crystals were in same polymorphic form in all of the samples (19). This is consistent with the SFC curves (Figure 3), which showed that all samples began to decrease in solid fat at the same temperature.

There was no significant difference in maximum peak temperature between the paired CLARSO/soy oil samples at oil levels of 60%, 65%, and 70%. The soy oil samples had significantly greater maximum temperatures than their paired CLARSO samples at oil

levels of 75% and 80%. As the amount of oil in the samples was increased, the maximum temperature decreased. This indicated that samples with greater amounts of solid fat melted at a higher temperature. The Crisco sample had a maximum temperature that was not significantly different from that of the 75% and 80% CLARSO samples and the PHO sample had a maximum temperature that was not significantly different from that of the 70% and 75% CLARSO and 80% soy oil samples.

There was no significant difference between the offset temperatures of the paired CLARSO/soy oil samples at oil levels of 60% and 65%. The soy oil samples had significantly greater offset temperatures than their paired CLARSO samples at oil levels of 70%, 75%, and 80%. There was no relationship observed between the percent of oil in the sample and the offset temperature. The Crisco sample had an offset temperature that was not significantly different from that of the 60%, 65%, and 75% CLARSO/soy oil samples, the 75% CLARSO sample, and the 80% CLARSO/soy oil samples. The PHO sample had an offset temperature that was significantly greater than that of any of the other samples.

At all oil levels except 65%, there was no significant difference between the enthalpy of the paired CLARSO/soy oil samples. The 65% CLARSO sample had a significantly higher enthalpy than the 65% soy oil sample. As the percent of oil in the samples was increased, the enthalpy was decreased, and therefore samples with more solid fats absorbed more heat upon melting. This result is consistent with that of the crystallization cycles: samples with greater amounts of saturated fatty acids absorbed/released more

heat upon melting/crystallization. The Crisco sample had an enthalpy between that of the 65% soy oil sample and the 70% CLARSO/soy oil samples. The PHO sample had an enthalpy that was not significantly different from that of the 65% CLARSO samples.

There were several conclusions drawn from these DSC results. Shortening samples with more solid fats crystallized and melted at higher temperatures than those with less solid fats. The type of oil used (CLARSO or soy oil) did not affect the crystallization temperature of the shortenings. The CLARSO absorbed more heat per gram upon crystallization than did the soy oil, indicating that it had a higher crystalline fraction, which was consistent with the results of the strain and frequency sweeps and SFC. The types/amounts of fatty acids present in the shortening samples did not influence the isothermal crystallization time, and it was proposed that other factors, such as ambient temperature and container geometry, play a more significant role in this process. All samples began melting at the same temperature, which was consistent with the SFC findings.

Microstructure

Figure 1.7 shows microscopic images of the shortening samples under polarized light. There was no observable difference between the CLARSO and soy oil samples that had the same amount of oil. It was proposed that this was due to the stearin having a greater effect on the structure of the shortenings than the liquid oil, a hypothesis supported by the results of the rheology and solid fat content. Marangoni *et. al.* (22) found that for edible crystal fat networks, the microstructure and SFC were the physical parameters most closely correlated to the G', and the relationship between the rheology, SFC, and

microstructure in this study supports that finding. As the amount of oil in the samples was increased, the fat crystals appeared more diluted in the liquid oil. The Crisco sample appeared to have smaller, more uniform crystals, a quality which was attributed to the standardized commercial process used to produce it.

CONCLUSION

In general, the CLA-rich shortening samples displayed improved physical properties relative to the corresponding soy oil samples. This finding is conclusive with those of Shah *et. al.* (15), who found that CLA-rich margarine provided a better texture relative to soy oil control margarine. CLA-rich shortenings had comparable rheological properties to those of commercially available shortenings, indicating that they could serve as a healthier alternative without compromising physical properties. The CLA-rich shortenings had significantly higher solid fat contents than soy oil controls only at higher oil levels, indicating that although the type of oil influenced the structure, the solid fat (stearin) played a more significant role. The type of oil used did not influence the crystallization temperature of the shortenings; however, the CLARSO shortenings displayed relatively higher crystalline mass fractions. This suggested that although CLARSO and soy oil crystallize at the same temperature, CLARSO interacts with stearin to produce a more stable crystal matrix, leading to the improved rheological properties and greater solid fat content. There were no visibly detectable differences in the microstructure of the paired CLA/soy oil shortenings, which supported the conclusion previously drawn from the rheology results that the stearin contributed more significantly to the crystal structure of the shortening than did the oil. In summary, CLARSO produced firmer shortenings than

did conventional soy oil by interacting with the crystallizing stearin fraction and consequently increasing the crystalline mass fraction without significantly altering the microstructure kinetics of solid fat crystallization.

The commercial control samples (Crisco and PHO) were comparable to the CLA and soy oil shortenings. However, there was not a detectable consistency among the rheology, thermal behavior, and microstructure concerning exactly which CLARSO or soy oil sample coincided the most with either of the controls. The Crisco sample displayed a more uniform crystal structure and its rheological properties were comparable to the 65% soy oil sample. However, it displayed SFC results comparable to the 70% soy oil sample. It was proposed that the standardized industrial process (involving a controlled temperature-time profile) used to produce Crisco created a more solid product without adding additional solid fats. Therefore, if this industrial process was used to produce CLA-rich shortenings, a higher oil to solid fat ratio could be employed, which would increase the amount of CLA delivered and decrease the amount of saturated hard fats.

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Table 1.1- Samples of CLA-rich shortening and soy oil controls prepared.

Sample	Amount CLARSO (g)	Amount stearin (g)	Sample	Amount soy oil (g)	Amount stearin (g)
60% CLARSO	240	160	**60% soy oil**	240	160
65% CLARSO	260	140	**65% soy oil**	260	140
70% CLARSO	280	120	**70% soy oil**	280	120
75% CLARSO	300	100	**75% soy oil**	300	100
80% CLARSO	320	80	**80% soy oil**	320	80

Table 1.2- Fatty acid data for CLARSO and soy oil used to produce shortenings. Samples were analyzed in duplicate and error indicates standard deviation. Statistical analysis was performed across rows to identify significant differences between individual fatty acids. Samples connected by same letter are not significantly different.

	CLARSO	Soy Oil
Fatty Acid	%	%
C16:0	13.54 ± 0.03a	13.98 ± 0.70a
C18:0	5.20 ± 0.03a	4.112 ± 0.80b
C18:1	26.78 ± 0.03a	23.25 ± 1.22b
C18:2	31.85 ± 0.03b	54.74 ± 1.58a
C18:3	1.80 ± 0.01b	5.41 ± 0.80a
CLA *cis,trans/trans,cis*	15.76 ± 0.41	-
CLA *trans,trans*	5.07 ± 0.41	-
Total CLA	20.83 ± 0.00	-
Total saturated fat	18.74 ± 0.04a	18.09 ± 1.07a

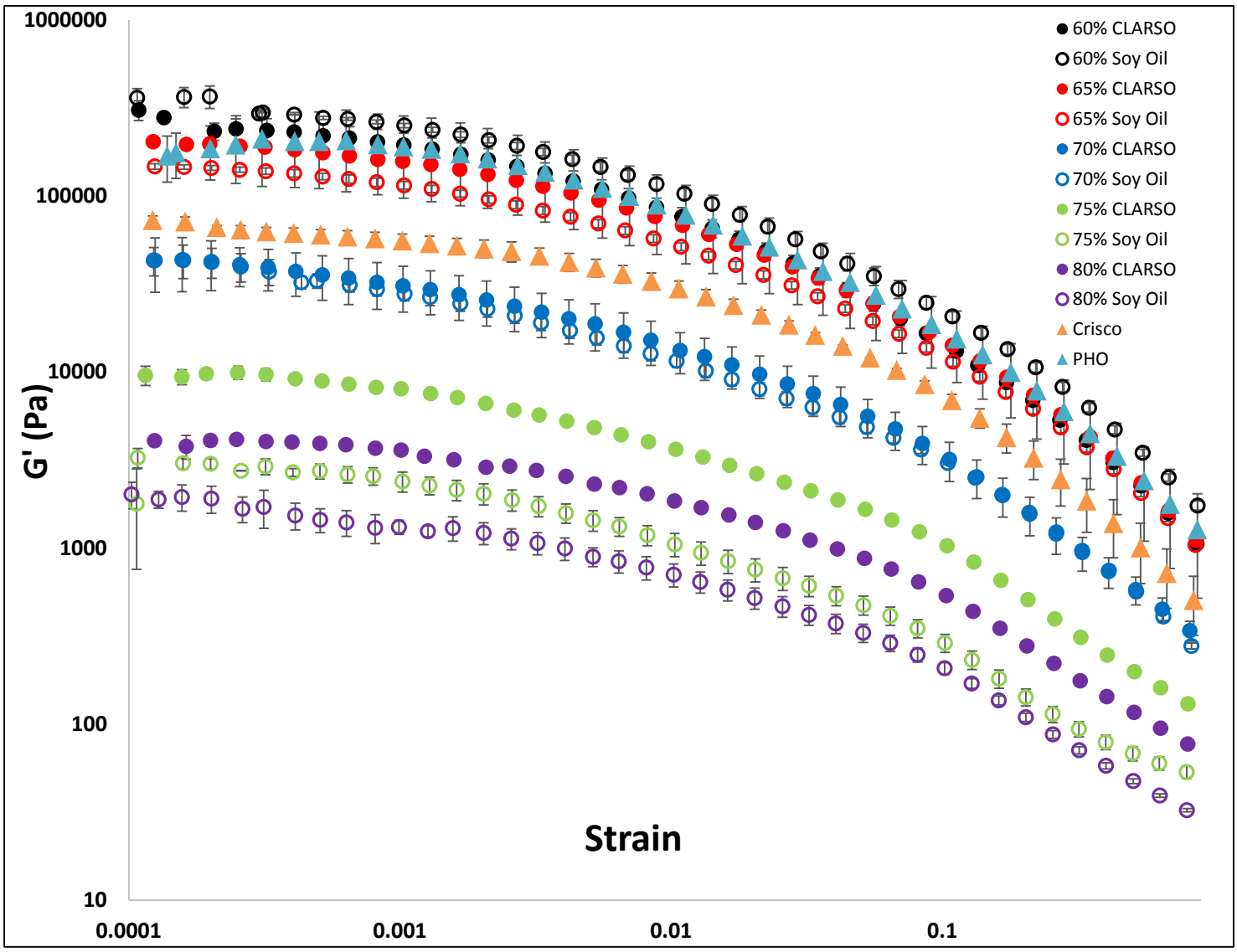


Figure 1.1. Elastic modulus (G') plotted as a function of percent strain for CLARSO, soy oil and commercial shortening samples. The analysis was performed in duplicate and the error bars represent one standard error from the mean.

Table 1.3- The Rheology strain sweep of shortening with various amounts of soy and CLA-rich soy oil. Analysis was performed in duplicate and error indicates one standard error from the mean. The overall means were transformed logarithmically to obtain a better comparison. Statistical analysis was performed among all samples. Samples with the same connecting letter are not significantly different.

Sample	Overall mean
60% CLARSO	11.77 ± 0.24b
60% Soy Oil	12.06 ± 0.21a
65% CLARSO	11.61 ± 0.19c
65% Soy Oil	11.30 ± 0.19d
70% CLARSO	9.93 ± 0.28f
70% Soy Oil	9.83 ± 0.22f
75% CLARSO	8.62 ± 0.18g
75% Soy Oil	7.40 ± 0.21i
80% CLARSO	7.87 ± 0.15h
80% Soy Oil	6.91 ± 0.18j
Crisco	10.67 ± 0.17e
PHO	11.67 ± 0.43bc

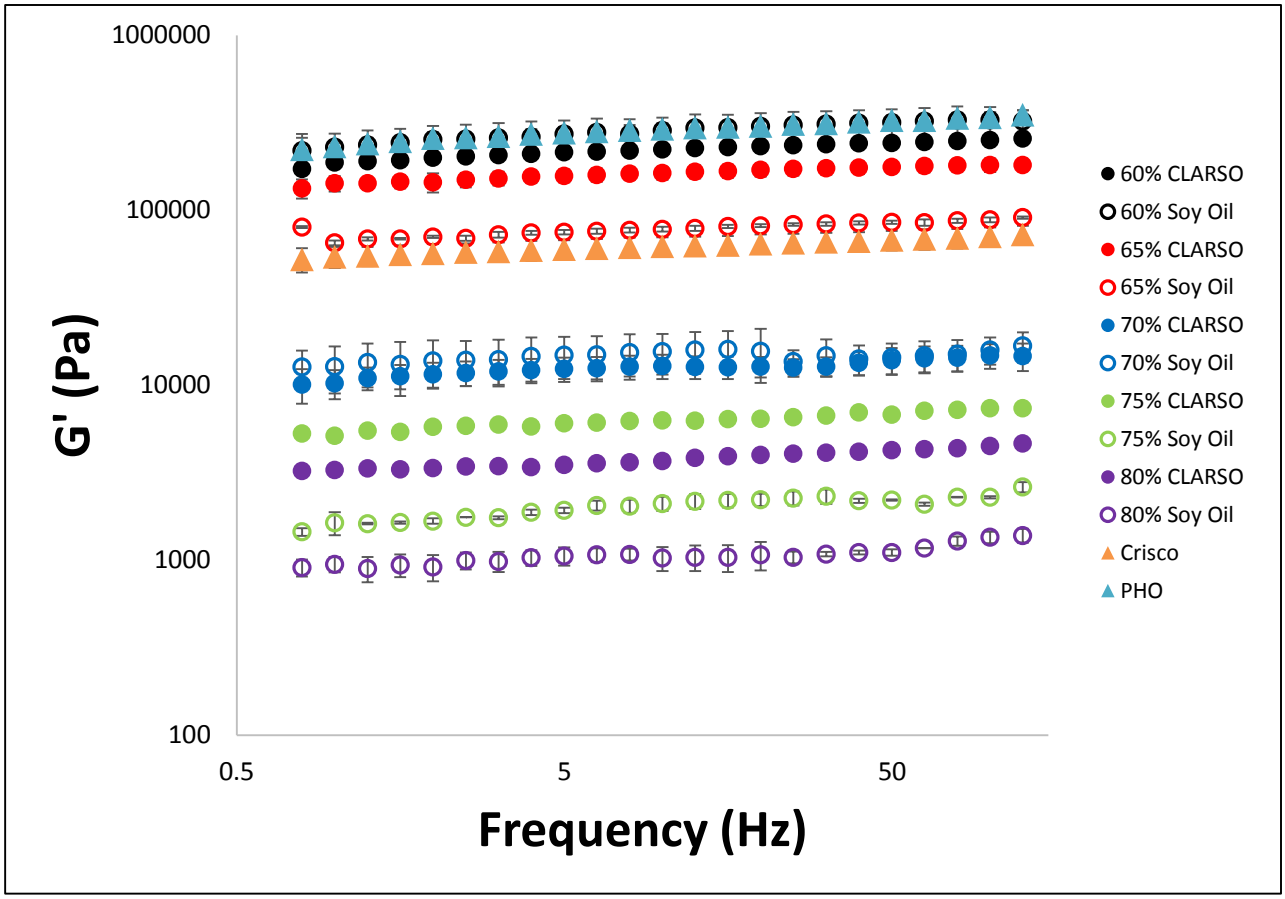


Figure 1.2. Elastic modulus (G') plotted as a function of angular frequency (rad/s) for CLARSO, soy oil, and commercial shortening samples. The analysis was performed in duplicate and the error bars represent one standard error from the mean.

Table 1.4- Overall means and connecting letters for frequency sweep results. Analysis was performed in duplicate and error indicates one standard error from the mean. The overall means were transformed logarithmically to obtain a better comparison. Statistical analysis was performed among all samples. Samples with the same connecting letter are not significantly different.

Sample	Overall mean
60% CLARSO	12.34 ± 0.02b
60% Soy Oil	12.56 ± 0.04a
65% CLARSO	12.01 ± 0.04c
65% Soy Oil	11.27 ± 0.05d
70% CLARSO	9.46 ± 0.03f
70% Soy Oil	9.60 ± 0.04f
75% CLARSO	8.82 ± 0.02g
75% Soy Oil	7.67 ± 0.03i
80% CLARSO	8.30 ± 0.02h
80% Soy Oil	7.08 ± 0.04j
Crisco	11.07 ± 0.02e
PHO	12.62 ± 0.03a

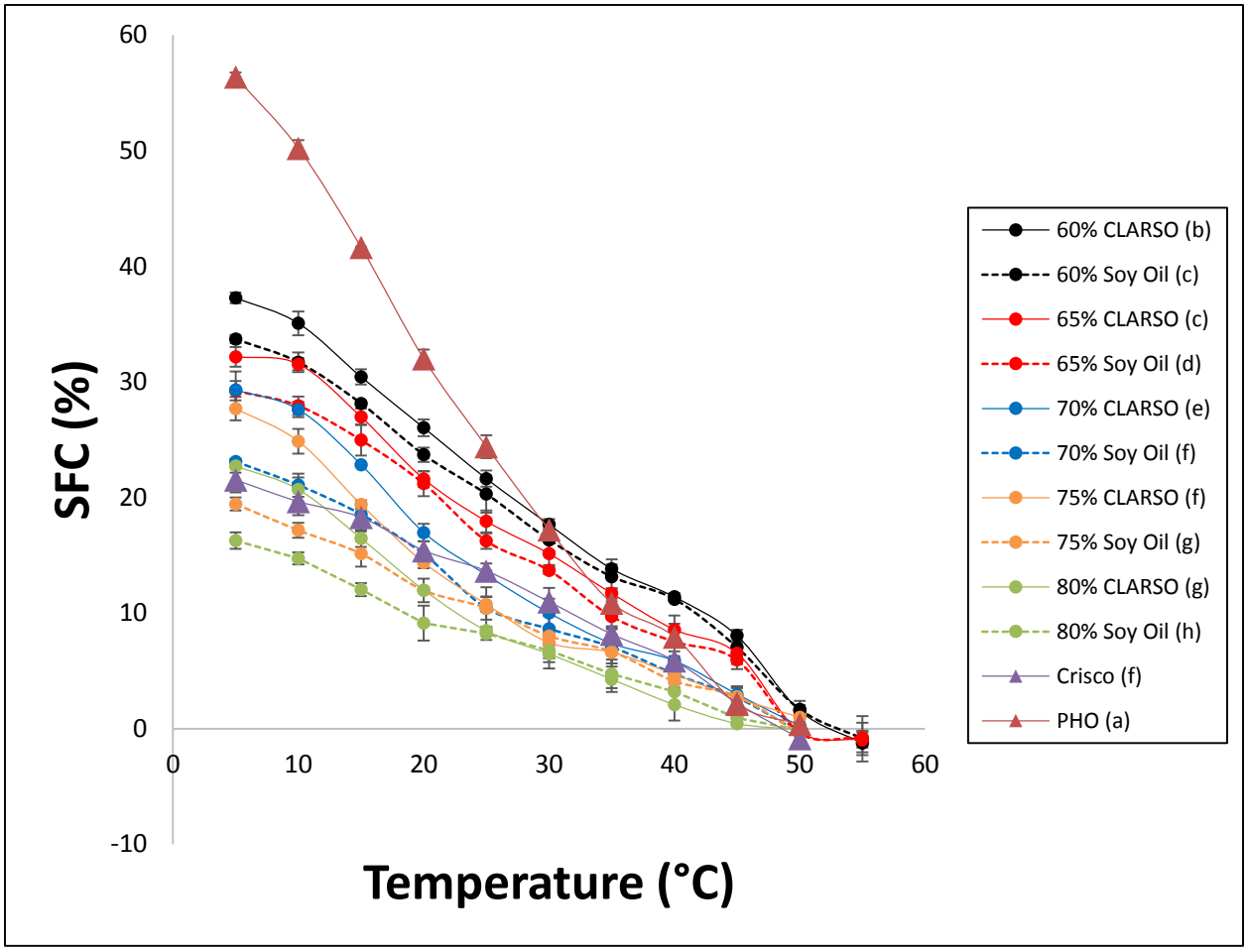


Figure 1.3. Solid fat content plotted as a function of temperature for CLARSO, soy oil, and commercial shortening samples. The analysis was performed in triplicate and the error bars indicate standard deviation.

Table 1.5- Overall means and connecting letters for SFC results. Analysis was performed in triplicate and error indicates one standard error from the mean. Statistical analysis was performed among all samples. Samples with the same connecting letter are not significantly different.

Sample	Overall mean
60% CLARSO	26.03 ± 0.42b
60% Soy Oil	23.70 ± 0.36bc
65% CLARSO	21.60 ± 0.38cd
65% Soy Oil	21.20 ± 0.62d
70% CLARSO	16.97 ± 0.44e
70% Soy Oil	15.10 ± 0.12ef
75% CLARSO	14.37 ± 0.27fg
75% Soy Oil	11.97 ± 0.59g
80% CLARSO	12.00 ± 0.15g
80% Soy Oil	9.13 ± 0.87h
Crisco	15.40 ± 0.47ef
PHO	32.00 ± 0.46a

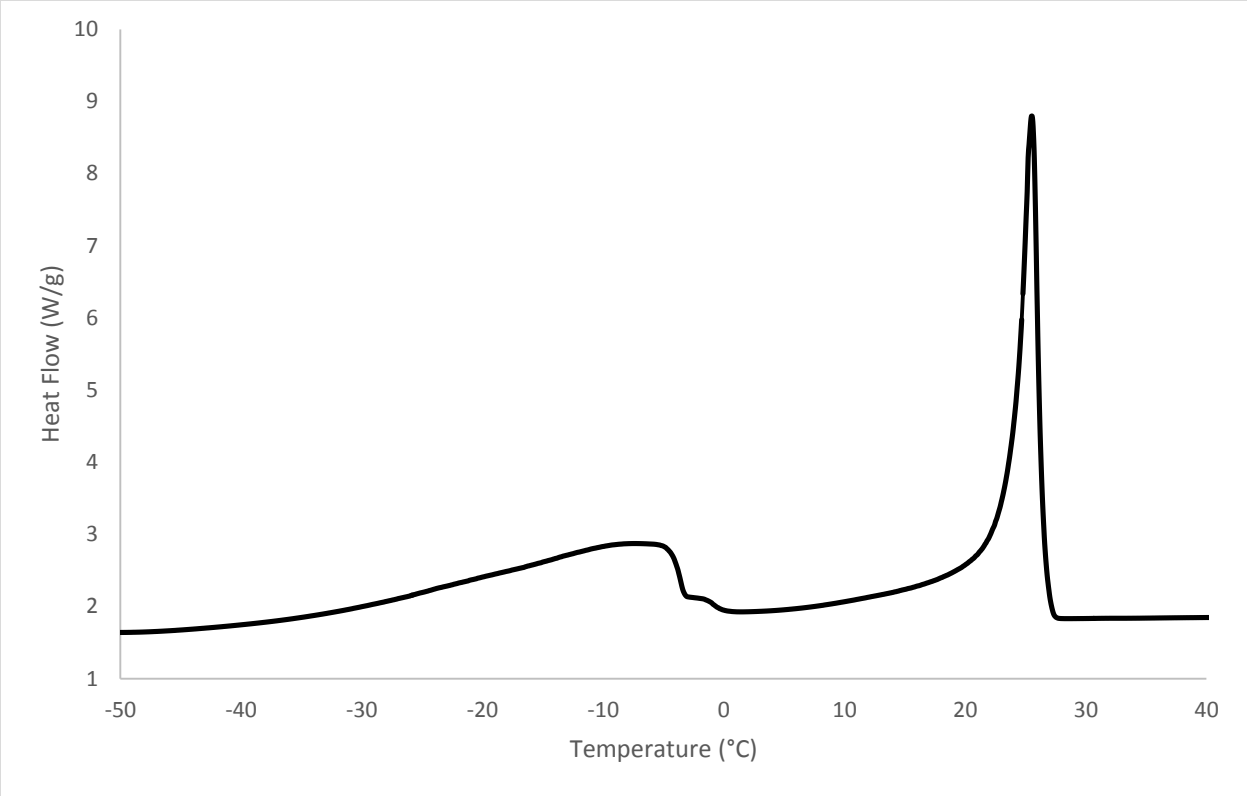


Figure 1.4. DSC spectrum for crystallization cycle of 60% CLARSO, plotted as heat flow (W/g) versus temperature (°C).

Table 1.6- DSC measurements from non-isothermal crystallization phase of DSC for CLA, soy oil, and commercial control samples. Analysis was performed in triplicate and error indicates standard deviation. Statistical analysis was performed within each column and samples with the same connecting letter are not significantly different.

Sample	Onset 1 (°C)	Max 1 (°C)	Onset 2 (°C)	Max 2 (°C)	Enthalpy 1 (J/g)	Enthalpy 2 (J/g)	Total Enthalpy (J/g)
60% CLARSO	26.96 ± 0.55cd	25.55 ± 0.13c	0.08 ± 0.34bcd	-7.57 ± 0.06b	23.83 ± 0.30a	23.94 ± 0.32d	47.77 ± 0.61bc
60% Soy Oil	27.89 ± 0.07bc	25.46 ± 0.18c	0.75 ± 0.03bc	-8.15 ± 0.22bc	23.89 ± 0.57a	20.41 ± 0.37ef	44.30 ± 0.94cd
65% CLARSO	25.91 ± 0.08ef	24.45 ± 0.00d	1.38 ± 0.48b	-8.01 ± 0.00bc	21.74 ± 0.07b	23.72 ± 0.05d	45.46 ± 0.02bcd
65% Soy Oil	26.77 ± 0.75de	23.79 ± 0.41d	-0.30 ± 0.77cd	-9.59 ± 0.93de	20.56 ± 1.16bc	19.49 ± 0.84ef	40.05 ± 2.00e
70% CLARSO	23.12 ± 0.03g	21.83 ± 0.21e	-0.94 ± 0.38de	-7.78 ± 0.36bc	16.77 ± 0.12d	32.35 ± 1.94b	49.12 ± 2.06b
70% Soy Oil	25.12 ± 0.15f	21.03 ± 0.57ef	-1.82 ± 0.17ef	-7.52 ± 0.85b	15.99 ± 1.07d	27.08 ± 1.40c	43.08 ± 2.19de
75% CLARSO	21.82 ± 0.24h	19.87 ± 0.23g	-2.34 ± 0.03ef	-8.92 ± 0.03bcd	14.96 ± 0.07d	33.97 ± 0.81b	48.93 ± 0.88b
75% Soy Oil	21.63 ± 0.38h	20.29 ± 0.30fg	-3.09 ± 0.55fg	-9.13 ± 0.18cde	12.28 ± 0.89e	19.42 ± 0.20f	31.71 ± 1.10f

80% CLARSO	19.85 ± 0.18i	18.00 ± 0.11h	-2.52 ± 0.79f	-10.47 ± 0.20e	11.95 ± 0.04e	32.29 ± 0.41b	44.24 ± 0.44cd
80% Soy Oil	22.57 ± 0.48gh	18.10 ± 0.73h	-4.10 ± 0.59g	-9.62 ± 0.90de	10.55 ± 0.68e	21.87 ± 0.36de	32.42 ± 1.02f
Crisco	31.51 ± 0.10a	29.47 ± 0.10a	-7.57 ± 0.19h	-18.24 ± 0.23f	19.11 ± 0.38c	12.48 ± 0.37g	31.59 ± 0.75f
PHO	28.91 ± 0.34b	27.80 ± 0.03b	12.60 ± 0.82a	2.19 ± 0.01a	15.60 ± 0.63d	59.50 ± 0.46a	75.10 ± 1.08a

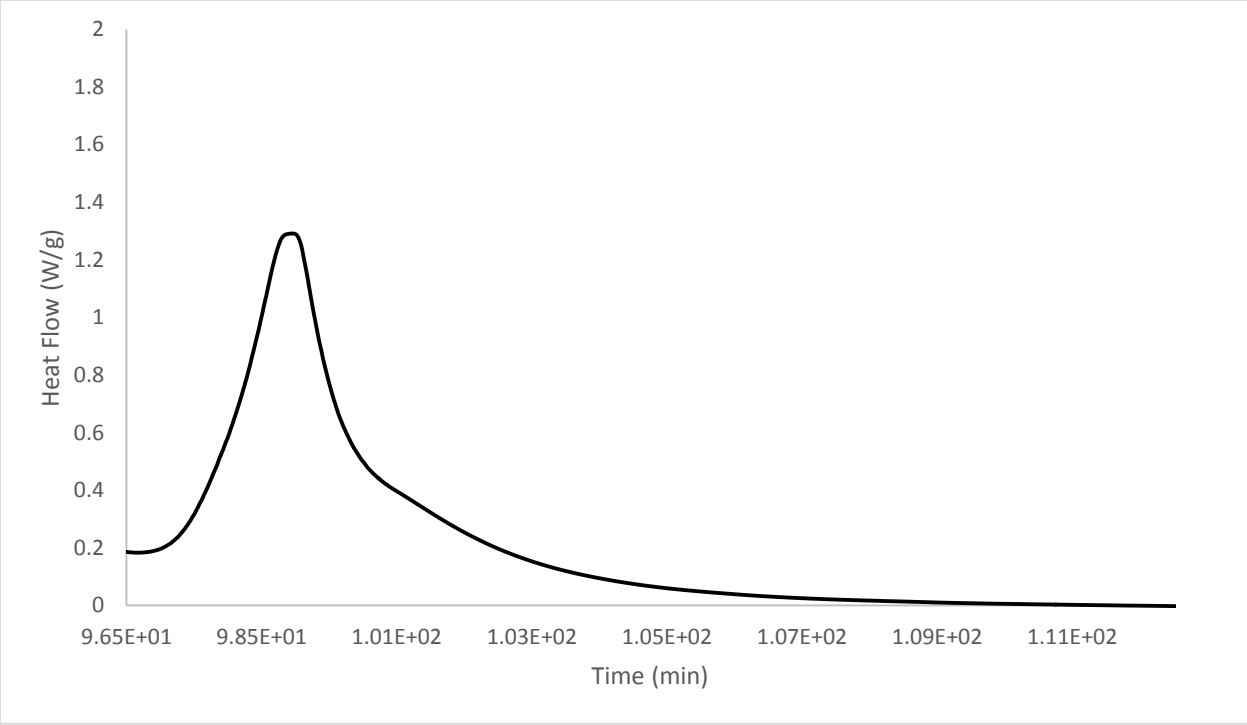


Figure 1.5. DSC spectrum for isothermal crystallization cycle of 60% CLARSO, plotted as heat flow (W/g) versus time (min).

Table 1.7- DSC measurements from isothermal crystallization phase of DSC for CLA, soy oil, and commercial control samples. Analysis was performed in triplicate and error indicates standard deviation. Statistical analysis was performed within each column and samples with the same connecting letter are not significantly different.

Sample	Onset	Max	Offset	Enthalpy
60% CLARSO	1.69 ± 0.03f	3.55 ± 0.04bcd	10.69 ± 0.63bcd	11.19 ± 0.22a
60% Soy Oil	1.51 ± 0.24f	2.22 ± 0.62d	7.66 ± 0.66ef	10.96 ± 0.49a
65% CLARSO	1.58 ± 0.04f	2.76 ± 1.01cd	10.37 ± 0.16cd	10.54 ± 0.01ab
65% Soy Oil	1.67 ± 0.02f	2.21 ± 0.48d	7.58 ± 0.85ef	9.38 ± 0.62b
70% CLARSO	2.54 ± 0.12c	5.19 ± 0.04b	12.34 ± 0.73b	7.03 ± 0.14c
70% Soy Oil	2.17 ± 0.16cde	3.06 ± 0.03bcd	7.64 ± 0.45ef	5.22 ± 0.72d
75% CLARSO	2.96 ± 0.12b	4.85 ± 1.63bc	12.45 ± 0.47b	5.09 ± 0.44d
75% Soy Oil	2.08 ± 0.20de	2.81 ± 0.20cd	7.02 ± 0.27f	5.72 ± 0.60d
80% CLARSO	4.08 ± 0.15a	7.04 ± 0.18a	13.29 ± 0.83a	3.25 ± 0.24e
80% Soy Oil	1.89 ± 0.17ef	3.12 ± 0.50bcd	6.99 ± 1.09f	2.84 ± 0.59e
Crisco	1.57 ± 0.08f	4.39 ± 0.22bcd	8.94 ± 0.71de	3.68 ± 0.24e
PHO	2.29 ± 0.02cd	4.04 ± 1.52bcd	11.41 ± 0.27abc	10.12 ± 0.27ab

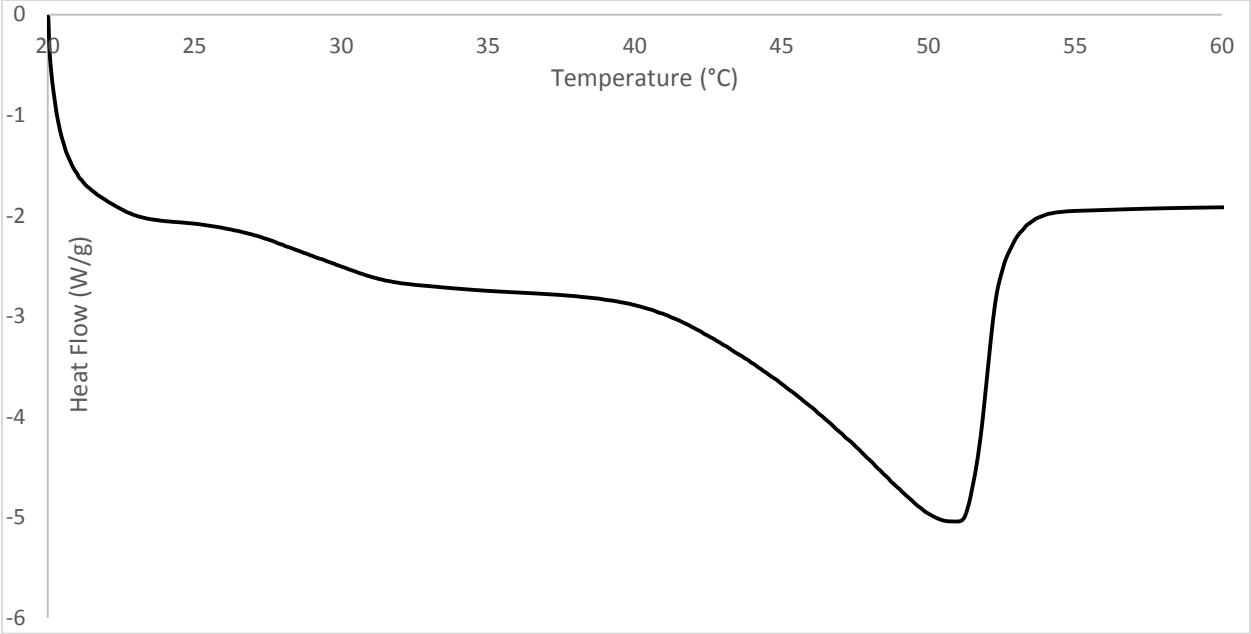
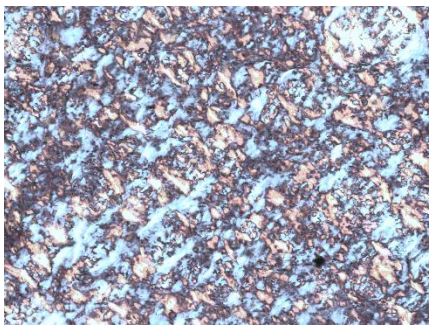


Figure 1.6. DSC spectrum for melting cycle of 60% CLARSO, plotted as heat flow (W/g) versus temperature (°C).

Table 1.8- DSC measurements and connecting letters from melting phase of DSC for CLA, soy oil, and commercial control samples. Analysis was performed in triplicate and error indicates standard deviation. Statistical analysis was performed within each column and samples with the same connecting letter are not significantly different.

Sample	Onset (°C)	Max (°C)	Offset (°C)	Enthalpy (J/g)
60% CLARSO	22.90 ± 0.41ab	50.90 ± 0.03a	54.22 ± 0.04bc	34.01 ± 0.78a
60% Soy Oil	23.01 ± 0.34ab	50.69 ± 0.04a	54.73 ± 0.44b	34.27 ± 0.39a
65% CLARSO	22.59 ± 0.05ab	50.01 ± 0.00a	54.34 ± 0.28bc	29.81 ± 0.14b
65% Soy Oil	22.95 ± 0.15ab	50.39 ± 0.26a	54.52 ± 0.06bc	26.02 ± 1.80c
70% CLARSO	22.06 ± 1.46b	47.38 ± 0.24bc	51.45 ± 0.71d	18.47 ± 0.88e
70% Soy Oil	24.01 ± 0.80ab	48.36 ± 0.85b	53.89 ± 0.90bc	17.48 ± 0.48e
75% CLARSO	23.46 ± 0.12ab	46.53 ± 0.09cd	52.59 ± 0.19cd	13.98 ± 0.16f
75% Soy Oil	23.59 ± 0.78ab	47.91 ± 0.61b	58.07 ± 1.40a	13.62 ± 0.32fg
80% CLARSO	23.97 ± 0.64ab	45.58 ± 0.16d	52.72 ± 0.69cd	10.49 ± 1.87h
80% Soy Oil	24.09 ± 0.97a	46.69 ± 0.38c	54.96 ± 0.18b	11.04 ± 0.59gh
Crisco	23.81 ± 0.29ab	45.55 ± 0.12d	53.06 ± 0.14bcd	21.67 ± 1.05d
PHO	23.95 ± 0.75ab	46.68 ± 0.01c	58.75 ± 1.17a	30.31 ± 0.95b



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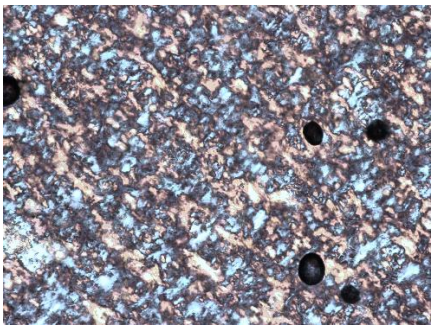

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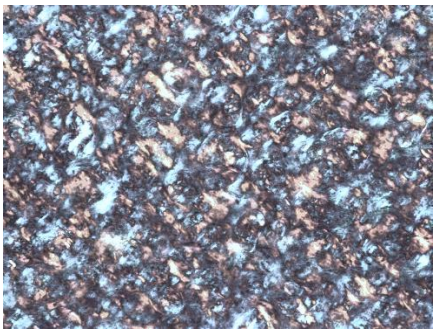

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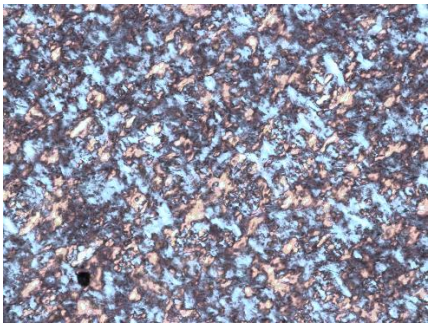

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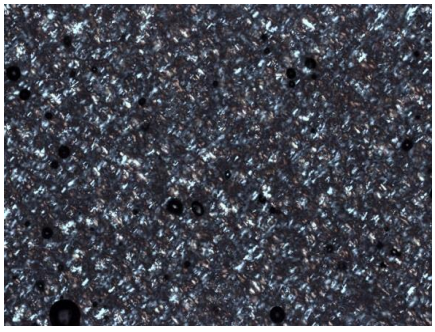

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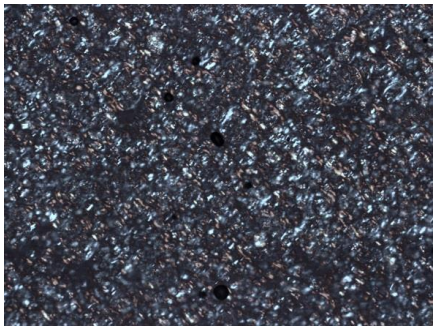

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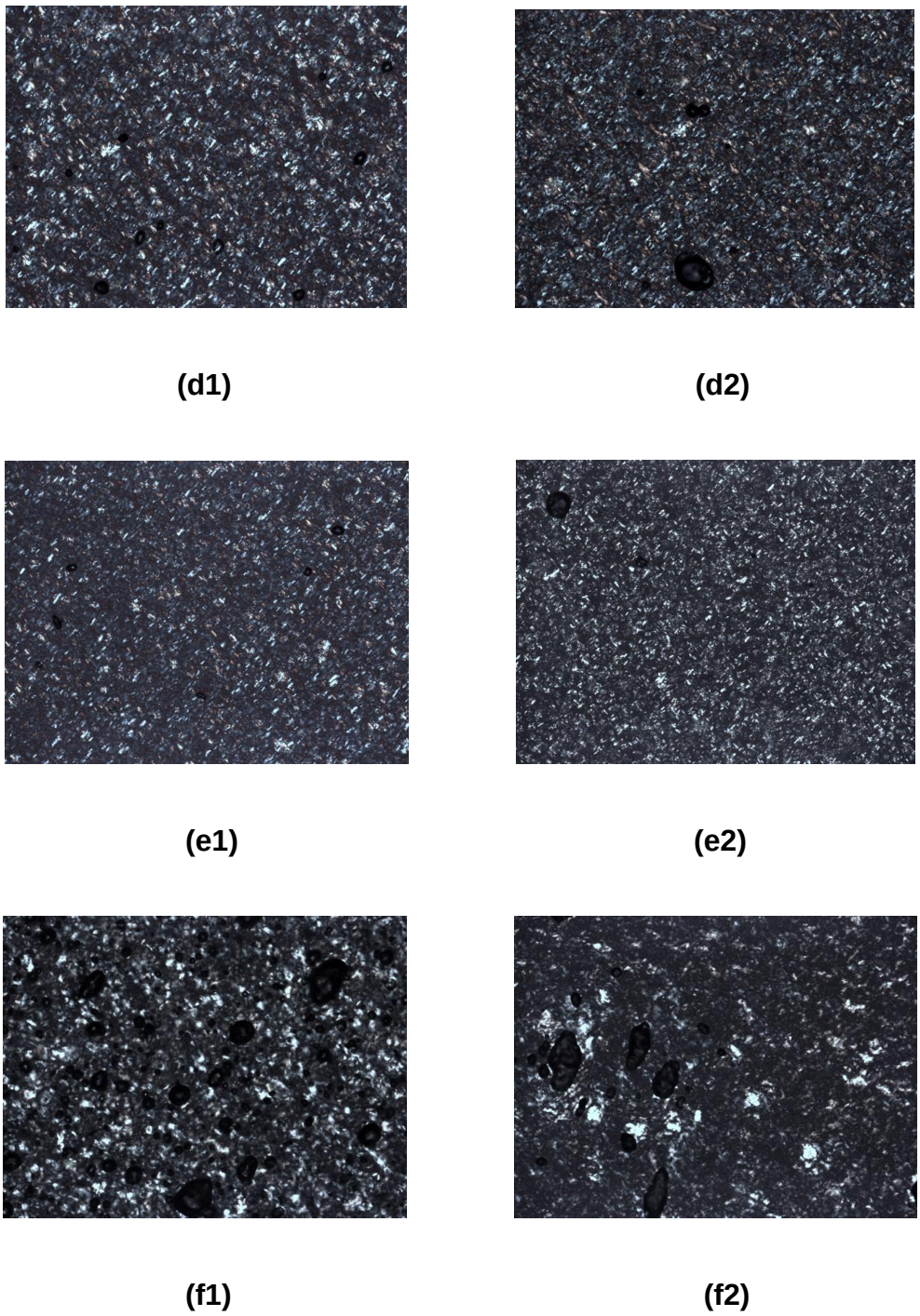

Figure 1.7. Microscope images of (a1) 60% CLARSO, (a2) 60% soy oil, (b1) 65% CLARSO, (b2) 65% soy oil, (c1) 70% CLARSO, (c2) 70% soy oil, (d1) 75% CLARSO, (d2) 75% soy oil, (e1) 80% CLARSO, (e2) 80% soy oil, (f1) Crisco, and (f2) PHO shortening samples observed under polarized light (scale width = 750 μm).

CHAPTER 2

CLA-rich chocolate paste and chocolate bar production and characterization.

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ABSTRACT

Conjugated linoleic acid (CLA) is an 18-carbon fatty acid with multiple health benefits, including anti-obesity and anti-carcinogenic properties. CLA-rich soy oil (CLARSO) can be produced through a heterogeneous catalysis process, and this oil was previously used to produce CLA-rich margarines and shortenings. The objectives of this study were to produce CLA-rich chocolate bars and pastes by replacing a portion of the fat with CLARSO and compare the rheological, textural, and thermal properties of these pastes/bars to controls made with either soy oil or traditional fats. CLARSO was used to prepare bars/pastes. Rheology, firmness, and thermal behavior of the pastes and fracturability, hardness, and thermal behavior of the bars were determined. The CLARSO chocolate pastes/bars contained no additional saturated fat relative to soy oil controls but the pastes had more solid rheology and were firmer and the bars had a higher fracture force relative to soy oil controls. Relative to non-soy controls, CLARSO pastes had similar rheology and CLARSO bars had similar fracturability, despite containing less saturated fat. The fat crystals of all samples were in the same polymorphic form. Therefore, CLARSO has the ability to produce chocolate pastes/bars with similar physical properties as traditional products containing more saturated fat.

Key Words

Conjugated linoleic acid-rich soy oil (CLARSO), chocolate paste, chocolate bars, rheology, texture, differential scanning calorimetry (DSC)

INTRODUCTION

Conjugated linoleic acid (CLA) is an 18-carbon dietary fatty acid with various positional and geometric isomers, found mostly in dairy and bovine foods as a product of rumen fermentation (1). CLA has many possible positive human health benefits, including anti-carcinogenic properties (2,3) and the ability to fight atherosclerosis (4,5), lower the risk of diabetes (6), and improve immune function (7). However, the typical diet provides only a fraction of the 3.2 grams of CLA needed daily to gain these health benefits, and increasing dietary CLA by increased consumption of bovine foods is unadvisable as they are also high in saturated fat and cholesterol (8,9). Therefore, a CLA-rich food source that is low in saturated fats and cholesterol would be desirable.

Soy oil contains approximately 50% linoleic acid (LA), a fatty acid with the potential to be isomerized to CLA, and could therefore be used to produce CLA-rich food products that are low in saturated fat and cholesterol (10). Jain and Proctor (11) developed a process to produce a 20% CLA-rich soy oil (CLARSO) through the photoisomerization of soy oil LA in the presence of an iodine catalyst. However, it was necessary to remove the iodine in this process, which therefore made it difficult to commercialize. Shah and Proctor (12) developed a heterogeneous catalysis process that produced 20% CLARSO in 2 hours without the use of solvents. A ruthenium-on-carbon catalyst is combined with soy oil in a high temperature, vacuum distillation technique, similar to the deodorization process routinely used in industry. Unlike the iodine-catalyzed photoisomerization process (11), the ruthenium catalyst can easily be centrifuged and filtered out of the oil (13).

Gilbert *et. al.* (7) showed that when CLARSO was fed to obese Zucker rats, their serum total cholesterol and LDL cholesterol levels were reduced by 50% relative to rats fed a control soy oil diet and their liver weight was reduced by 35% after 100 days. The CLARSO also increased the expression of the PPAR-γ gene, which increases fat metabolism and alleviates insulin resistance.

Ruan and Proctor (14) found that CLARSO had greater solid fat properties relative to conventional soy oil, including increased viscosity and a higher melting point temperature. The change in physical behavior of CLARSO was attributed to increased intermolecular hydrophobic interaction forces of the CLA conjugated double bonds, specifically the *trans-trans* fatty acids in CLARSO. A CLARSO margarine was developed by Shah *et. al.* (15) and the firmness, rheology, thermal behavior, and microstructure were compared to a soy oil control and a commercially available margarine. The CLARSO margarine was firmer, better able to tolerate high levels of stress without deformation, and had a higher solid fat content than the soy oil control, while having physical properties comparable to those of commercial margarine. Five typical servings of this margarine would provide the recommended daily value of CLA and 185 Calories. Mayfield *et. al.* (16) subsequently developed CLA-rich shortenings and compared their rheology, thermal properties, and microstructure to those of soy oil controls and commercially available shortenings. The CLA-rich shortenings possessed more solid-like rheological properties and had a more stable crystal structure, as indicated by DSC analysis, than did the soy oil controls. Furthermore, CLA shortenings also had similar physical properties to commercial controls. The results of the margarine and shortening studies illustrate the effectiveness

of CLARSO as a replacement for conventional saturated fats in food products, while providing additional health benefits.

Chocolate, like shortening, is a fat-based food whose physical properties are dependent upon its polymorphic crystal structure. Therefore, the oils and fats used to produce chocolate products have a significant effect on product quality. A recent European Parliament and Council directive mandated that no more than 5% of the cocoa butter in chocolate bars be replaced with an alternative fat to maintain the EU standard of identity (Directive 2000/36/EC, European Parliament and Council, June 23, 2000). It will be of interest to know how such a replacement of cocoa butter with CLARSO, and the replacement of palm oil with CLARSO in chocolate paste, will affect chocolate products.

Therefore, the objectives of this study were to:

4) Determine the functional physical properties of chocolate paste prepared by replacing 25% of a palm oil/canola oil mixture with CLARSO, relative to control bars obtained by replacing 25% of the palm/canola mixture with soy oil, and a control made solely with the palm/canola oil mixture.

5) Determine the functional physical properties of chocolate bars prepared by replacing 5% of the cocoa butter with CLARSO relative to control bars obtained by replacing 5% of the cocoa butter with soy oil, and a control made solely with cocoa butter.

EXPERIMENTAL PROCEDURES

Materials

Refined, bleached, and deodorized (RBD) soy oil was obtained from Riceland Foods (Stuttgart, AR, USA). Cocoa butter, cocoa mass, soy lecithin, crushed sugar (Barry Callebaut, Wieze, Belgium), cocoa powder (Cargill, Wormer, The Netherlands), skim milk powder (Friesland, Campina, Belgium), palm oil, canola oil (Vandemoortele R&D, Izegem, Belgium), Palsgaard Oil Binder, Palsgaard PGPR 4150, and Palsgaard AMP 448 (Palsgaard A/S, Denmark) were used to produce chocolate bars and pastes at the University of Gent Cacaolab (Gent, Belgium). All chemicals used were reagent grade.

Methods

CLA-rich soy oil production and analysis

The heterogeneous catalytic process of Shah and Proctor (12) was adopted to produce CLA-rich soy oil from RBD soy oil, which was used to produce chocolate pastes and bars. The method of Lall *et. al.* (17) was used to determine the fatty acid profile of RBD and CLARSO duplicate samples as FAMES. Each sample was analyzed by GC-FID.

Chocolate paste production and analysis

Chocolate Paste Preparation: Chocolate pastes were prepared according to the method of Patel *et. al.* (18). There were three types of chocolate paste produced: CLARSO, soy oil control, and non-soy oil control. Each paste contained 30% (wt.) of a fat blend, the composition of which differed based on the type of paste. The fat blend used for the control paste consisted of 70% palm oil and 30% canola oil. The fat blends used for the

CLA-rich and soy oil pastes were prepared by making the control fat blend (70% palm oil and 30% canola oil) and then replacing 25% of this with either CLARSO or soy oil. The fat blends prepared are shown in Table 2.1.

One kilogram of each type of chocolate paste was prepared with these fat blends, as described in Table 2.2. Eighty percent (wt/wt) of the fat blend and the Palsgaard Oil Binder were combined in a Stephan UMC 5 mixer (Stephan Machinery, Hameln, Germany) set to a temperature of 60°C and stirred until the Oil Binder was completely dissolved. The milk powder, cocoa powder, and sugar were then added to the mixture which was stirred for approximately 2 minutes. The particle size of the mixture was reduced through refining using an Exakt 80S 3-roll mill (Exakt Technologies Inc., USA) with a roll temperature of 35°C, a roll distance of 3-1, and a speed of 400 rpm. The refined mixture was placed back into the Stephan Mixer, the remaining fat blend, Palsgaard PGPR 4150, and Palsgaard AMP 4448 were added, and the mixture was stirred for approximately 2 minutes. The chocolate paste was transferred into 20 cylindrical plastic containers (1 in. diameter x 1 in. height) for texture analysis and into 6-50 mL centrifuge tubes for rheology and thermal analysis. Pastes were stored at 20°C for one week prior to analysis.

Rheology Determination: The rheology of the pastes was determined in sample duplicate after one week of storage at 20°C (deemed “week 1”) and after two weeks of storage at 20°C (deemed “week 2”) with an AR 2000 Rheometer (TA Instruments, New Castle, DE, USA). Strain and frequency sweeps were performed to determine gel strength and solid-like behavior of the samples. A parallel plate with a cross-hatched geometry (diameter = 40 mm) was used with a geometry gap set at 1000 μm. The

strain sweep involved increasing the strain from 0.0001 to 100 while keeping the temperature constant at 20°C. The complex modulus G' was measured as a function of strain to analyze the resistance of the samples to deformation as there was increased stress applied to the sample. The frequency sweep involved increasing the frequency from 0.1 to 100 Hz while keeping the temperature at 20°C. G' was measured as a function of angular frequency to determine at what frequency the structure of the samples began to break down.

Firmness Analysis: Firmness analysis was performed on chocolate paste samples after one and two weeks of storage at 20°C. Five sample replicates were analyzed using a 5942 Instron TA 500 Texture Analyzer (Lloyd Instruments, Bognor Regis, West Sussex, UK). Firmness was defined as the force required to penetrate the samples using an 11 mm diameter cylindrical probe which entered the samples to a depth of 10 mm at a rate of 10 mm/min with a 0.1 N trigger value.

Thermal Analysis: Fat blends as used in each chocolate paste sample were prepared as shown in Table 2.3. The melting behavior of triplicate samples was determined by differential scanning calorimetry (DSC) using a Q1000 Tzero DSC (TA Instruments, New Castle, DE, USA). Indium (enthalpy and temperature), azobenzene (temperature), and undecane (temperature) were used to calibrate the instrument. The instrument was purged with nitrogen and an empty pan was used as reference. Five to ten mg of each fat blend was placed in hermetic aluminum pans and the pans were sealed. The melting behavior of the samples was determined by equilibrating the pans at 20°C for 10 minutes and then increasing the temperature at a rate of 5°C/min to a final temperature of 70°C. The temperature was measured as a function of heat flow and the

resulting DSC curves were analyzed using the Universal Analysis software (TA Instruments, New Castle, DE). A single inverted peak was observed and four parameters of this peak were analyzed: the onset temperature, the temperature at which the maximum heat flow was observed (the peak temperature), the offset temperature, and the enthalpy absorbed (the peak integration).

Chocolate bar production and analysis

Chocolate Bar Preparation: Chocolate bars were prepared according to the formulation in Table 2.4. Three types of bars were prepared: CLARSO, soy oil control, and non-soy oil control. The non-soy oil control was made solely with cocoa butter, and for the other samples 5% of the cocoa butter was replaced with either CLARSO or soy oil in the experimental samples. Two kg of each formulation were prepared according to the method of De Clercq *et. al.* (19). The cocoa butter was melted and the fat blend was prepared for each batch using the appropriate proportions of cocoa butter and/or CLARSO and soy oil for each formulation. The sugar, cocoa mass, and a portion of the fat blend (to attain a fat content of 27%) were mixed in a Vema mixer BM 30/20 (Vema Construct, NV Machinery, Verhoest, Izegem, Belgium). The particle size was reduced by refining with an Exakt 80S 3-roll mill (Exakt Technologies Inc., USA) with a roll temperature of 35°C. The refined mixture was conched in a Bühler Elk'olino Conche (Richard Frisse GmbH, Bad Salzuflen, Germany). There were two phases of conching: a dry phase and a liquid phase. The dry phase consisted of stirring the mixture clockwise at a speed of 1200 rpm and a temperature of 55°C for 120 minutes. A shearing step was then performed by shearing the mixture anticlockwise at 1200 rpm and 65°C for 240 minutes. The lecithin and remaining fat blend were added and the liquid conching phase

was performed. The mixture was stirred at 1500 rpm and 45°C for 60 minutes and then a shearing step like that done in dry conching was performed. The conched chocolate was then tempered by hand. Approximately 2/3 of the chocolate was poured onto a chilled marble plate and handled manually until the viscosity had increased an appropriate amount and the chocolate reached a temperature of approximately 28°C. This chocolate was added back to the remaining 1/3 to melt any unstable crystals. The tempered chocolate was molded into chocolate bars (102 x 23 x 10 mm dimensions; Chocolate World, Antwerp, Belgium), cooled at 12°C for 4 hours, and then stored at 20°C for one week prior to analysis.

Fracturability Analysis: To analyze the fracturability of the bars, a three-point fracture test was performed with a TA 500 Texture Analyzer (Lloyd Instruments Ltd., West Sussex, UK) fitted with a 500 N load cell and a plastic cutting probe. Ten sample replicates were analyzed for each type of bar. The fracturability was defined as the maximum load (N) necessary to fracture a bar of chocolate. The probe descended at a rate of 2 mm/s with a trigger value of 0.2 N. The bar was considered fractured when the load dropped abruptly dropped by 25%.

Hardness Analysis: The hardness of 10 bars from each formulation was analyzed using the TA 500 Texture Analyzer (Lloyd Instruments Ltd., West Sussex, UK) fitted with a 500 N load cell and a needle-shaped probe. The hardness was defined as the maximum load (N) achieved when the probe penetrated the sample. The probe descended at a rate of 2 mm/s with a trigger value of 0.2 N, and entered the sample to a depth of 5 mm before retracting.

Thermal Analysis: Fat blends, as used in each chocolate bar sample, were prepared as shown in Table 2.5. The melting behavior of triplicate samples was determined using the DSC analysis procedure as previously described for the chocolate paste samples.

Data Analysis

All statistical analyses were performed using JMP 10 (SAS Institute, Inc., Cary, NC) statistical software. The fatty acid composition, rheology, texture, and thermal results were analyzed by comparing the overall means in a one-way ANOVA using Tukey's HSD test with an α-level of 0.05. The strain and frequency sweep G' values were transformed logarithmically to obtain a better comparison.

RESULTS AND DISCUSSION

CLA-rich soy oil fatty acid analysis

Table 2.6 shows the fatty acid data from FAMEs analysis of CLARSO and soy oil used to produce chocolate bars and pastes. There was no significant difference between the total saturated fat content of CLARSO and soy oil. The CLARSO contained 15.76% *cis,trans/trans,cis* CLA and 5.07% *trans,trans* CLA, with a total CLA content of 20.83%. Therefore, 15.4 g of this oil would need to be consumed daily in order to receive the recommended 3.2 g.

Chocolate Paste Characterization

Rheology Determination

Figure 2.1 shows the elastic modulus (G') plotted as a function of percent strain for the CLARSO, soy oil, and control chocolate pastes. All samples had similar linear

viscoelastic regions, between % strain values of 0.01 and 0.1. All samples also had a similar critical strain value required to deform the paste structure of approximately 0.2. Therefore, the microstructures of all samples were similarly resistant to deformation under increasing strain. The mean G' values for one decade of % strain, from 0.01 to 0.1, were compared to identify any statistically significant differences in solid-like behavior among samples, as shown in Table 2.7. For both the CLARSO and soy oil samples, the week 2 samples had significantly higher G' values than their respective week 1 values. Therefore, post-production isothermal crystallization and hardening occurred in both of these samples, increasing their solid-like behavior. There was no significant difference between the control week 1 and week 2 samples. The CLARSO week 1 and week 2 samples had significantly higher mean G' values than the soy oil week 1 and week 2 samples, respectively. This indicated that CLARSO provided the chocolate paste with a more solid crystal structure, without the addition of saturated fats. The CLARSO week 2 sample was not significantly different from the control week 2 sample. The control sample contained a greater portion of solid fat but had solid behavior similar to that of the CLARSO sample. Therefore, CLARSO behaved more like a solid fat without contributing additional saturated fatty acids.

Figure 2.2 shows G' as a function of increasing angular frequency for the CLARSO, soy oil, and control chocolate pastes. The % strain was held constant at 0.04 since samples had not significantly deformed at this strain value, as evidenced by the strain sweep. The curves for all samples had similar linear shapes, indicating that G' was independent of frequency at this strain value. Therefore, all samples behaved as solids below their critical strain value. The overall mean G' values were compared to determine any

statistically significant differences among samples, as shown in Table 2.8. The CLARSO week 2 sample had a significantly higher mean G' value than did the CLARSO week 1 sample. However, the control and soy oil week 2 samples had significantly lower mean G' values than did their respective week 1 samples. Therefore, the CLARSO sample displayed post-production isothermal crystallization hardening in this case but the control and soy oil samples did not. Although the CLARSO and soy oil week 1 samples were not significantly different, the CLARSO week 2 sample had a significantly higher G' value than did the soy oil week 2 sample. This was consistent with the strain sweep results: the CLARSO provided a more solid-like structure without contributing additional saturated fats.

Firmness Analysis

Figure 2.3 and Table 2.9 show the maximum force achieved when samples of CLARSO, soy oil, and control chocolate pastes were penetrated with a cylindrical probe, as an indication of the firmness of the samples. Measurements were taken after one and two weeks of storage at 20ºC. All week 2 samples showed significantly greater firmness than their respective week 1 samples. This indicates that significant amounts of post-production isothermal crystallization/hardening occurred, which is consistent with the results of the strain sweep analysis. Both the CLARSO week 1 and week 2 samples were significantly firmer than their respective soy oil samples. This was attributed to the ability of CLARSO to provide the samples with a more solid-like structure without the addition of saturated fats, as also seen in the rheology results. The control week 1 and week 2 samples were significantly firmer than the respective CLARSO and soy oil samples, indicating that although CLARSO offered improved

texture over soy oil, it did not behave similar to the more saturated palm oil with regards to texture.

Thermal Analysis

Figure 2.4 shows the DSC spectrums for CLARSO, soy oil, and control chocolate paste fat blends. Table 10 shows the values and statistical comparisons for the onset temperature, peak temperature, offset temperature, and enthalpy of the CLARSO, soy oil, and control chocolate paste fat blends. There were no significant differences in melting onset temperatures among any of the samples. This indicated that the fat crystals were in the same polymorphic form in all of the samples (20). There was no significant difference between the peak melting temperature of the CLARSO and soy oil fat blends. However, the control sample had a significantly higher peak temperature. This result was expected due to the greater amount of saturated fat in the control fat blend. There were no significant differences in offset temperature among any of the samples, indicating that their most saturated fraction, the palm oil, finished melting at the same temperature, as was expected. No significant differences in enthalpy were observed among any of the samples. This result illustrated that all samples had similar levels of thermodynamic stability and similar solid-like crystal matrices (21). Addition of CLARSO did not significantly affect the thermodynamic stability and solid behavior of the sample relative to the control fat blend. This is consistent with the strain sweep results and again indicated the potential of CLARSO to replace more saturated fats.

Chocolate Bar Characterization

Fracturability Analysis

Figure 2.5 and Table 2.11 show the force required to fracture CLARSO, soy oil, and control chocolate bars. The CLARSO bars had a significantly higher fracture force than did the soy oil bars, indicating that CLARSO provided a more solid texture without contributing additional solid fats. The fracture force of the CLARSO bars was not significantly different from that of the control bars. Therefore, the addition of CLARSO did not affect the solidity of the fat crystal matrix relative to that of the control bars made purely with cocoa butter. This result was consistent with those of the chocolate paste strain sweep and DSC analyses: CLARSO provided a more solid structure without containing additional saturated fats.

Hardness Analysis

Figure 2.6 and Table 2.12 show the maximum force achieved when CLARSO, soy oil, and control chocolate bars were punctured with a needle-shaped probe. There was no significant difference in the hardness of the CLARSO and soy oil bars. The control bar had a significantly higher hardness than the CLARSO and soy oil bars, although the CLARSO readings were most consistent. These results differed from those of the fracturability analysis, most likely because hardness is a function of density whereas fracturability is a function of geometry.

Thermal Analysis

Figure 2.7 shows the DSC spectrums of CLARSO, soy oil, and control chocolate bar fat blends. Table 2.13 shows the values and statistical comparisons for the onset temperature, peak temperature, offset temperature, and enthalpy of the CLARSO, soy oil, and control chocolate bar fat blends. The control fat blend had a significantly higher onset temperature than did the CLARSO and soy oil fat blends. This indicated that the lowest melting fraction of the control fat blend was more saturated than that of the CLARSO or soy oil fat blends. This was expected since the control blend contained purely solid fat whereas the other samples contained a portion of oil. However, there were no significant differences in peak temperature or offset temperature among any of the samples. This indicated that the fat crystals were in the same polymorphic form in all of the samples (20). There was no significant difference in enthalpy among the CLARSO and soy oil fat blends. However, the non-soy oil control fat blend had a significantly lower enthalpy than the CLARSO and soy oil blends.

CONCLUSION

Although the CLARSO paste and bars contained no additional solid fats in relation to the soy oil paste/bars, they displayed more solid-like physical properties. The CLARSO paste had greater rheological and textural properties relative to the soy oil control and the CLARSO bars had a greater fracture force than soy oil controls. Therefore, CLARSO provided samples with a more solid crystalline matrix without actually containing additional saturated fat.

Thermal analysis indicated that all samples displayed the same polymorphic crystal form, despite containing different fat blends. Therefore, partial replacement of palm oil and cocoa butter with CLA-rich and conventional soy oils did not change the form in which the fats crystallized.

The CLARSO paste and bars displayed similar physical properties relative to control paste/bars. The rheology and crystalline thermodynamic stability of the CLARSO paste was similar to that of the control paste and the CLARSO bars had a similar fracture force relative to the control bars. Therefore, the solidity of the fat crystal matrices of the CLARSO and control chocolate pastes and bars were similar despite the control samples containing more solid fats than the CLARSO samples. This demonstrated the ability of CLARSO to replace more saturated fats without compromising solid behavior.

The typical serving size for both chocolate pastes and bars is approximately 40 g. To obtain the recommended daily value of CLA (3.2 g), it would be necessary to consume 5 servings of chocolate paste or 66 servings of chocolate bars. Although 5 servings of chocolate paste per day is not unrealistic, 66 servings of chocolate bars is. However, to maintain the standard of identity of the bars, only 5% of the cocoa butter can replaced. Therefore, to produce chocolate bars that are a good source of CLA, the standard of identity could be abandoned to create a “chocolate product” rather than a chocolate bar.

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Table 2.1- Fat blends prepared for the production of CLA-rich, soy oil, and control chocolate pastes.

Oil	**CLARSO paste**	**Soy oil paste**	**Control paste**
	Amount	Amount	Amount
Palm oil	52.5%	52.5%	70%
Canola oil	22.5%	22.5%	30%
CLARSO	25%	---	---
Soy oil	---	25%	---

Table 2.2- Recipe overview for the production of CLA-rich, soy oil, and control chocolate pastes.

Ingredient	CLARSO paste		Soy oil paste		Control paste	
	% By mass	Amount (g)	% By mass	Amount (g)	% By mass	Amount (g)
Palm oil	15.75%	157.5	15.75%	157.5	21.0%	210.0
CLARSO	7.5%	75.0	---	---	---	---
Soy oil	---	---	7.5%	75.0	---	---
Canola oil	6.75%	67.5	6.75%	67.5	9.0%	90.0
Palsgaard Oil Binder	1.50%	15.0	1.50%	15.0	1.50%	15.0
Crushed sugar	47.85%	478.5	47.85%	478.5	47.85%	478.5
Skim milk powder	14.0%	140.0	14.0%	140.0	14.0%	140.0
Cocoa powder	6.0%	60.0	6.0%	60.0	6.0%	60.0
Palsgaard PGPR 4150	0.15%	1.5	0.15%	1.5	0.15%	1.5
Palsgaard AMP 4448	0.5%	5.0	0.5%	5.0	0.5%	5.0

Table 2.3- Fat blends used for the thermal analysis of chocolate pastes.

Sample	Amount palm oil (g)	Amount canola oil (g)	Amount CLARSO (g)	Amount soy oil (g)	Total amount (g)
CLARSO Paste	26.25	11.25	12.5	---	50
Soy Oil Paste	26.25	11.25	---	12.5	50
Control Paste	35	15	---	---	50

Table 2.4- Recipe overview for the production of CLA-rich, soy oil, and control chocolate bars.

Ingredient	CLARSO bars		Soy oil bars		Control bars	
	% By mass	Amount (g)	% By mass	Amount (g)	% By mass	Amount (g)
Cocoa butter	11.02%	220.4	11.02%	220.4	11.6%	232.0
CLARSO	0.58%	11.6	---	---	---	---
Soy oil	---	---	0.58%	11.6	---	---
Crushed sugar	48.0%	960.0	48.0%	960.0	48.0%	960.0
Cacao mass	40.0%	800.0	40.0%	800.0	40.0%	800.0
Soy lecithin	0.4%	8.0	0.4%	8.0	0.4%	8.0

Table 2.5- Fat blends used for thermal analysis of chocolate bars.

Sample	Amount cocoa butter (g)	Amount CLARSO (g)	Amount soy oil (g)	Total amount (g)
CLARSO Bars	47.5	2.5	---	50
Soy oil Bars	47.5	---	2.5	50
Control Bars	50	---	---	50

Table 2.6- Fatty acid data for CLARSO and soy oil used to produce chocolate pastes and bars. Samples were analyzed in duplicate and error indicates standard deviation. Statistical analysis was performed across rows to identify significant differences between individual fatty acids. Samples connected by same letter are not significantly different.

	CLARSO	Soy Oil
Fatty Acid	%	%
C16:0	13.54 ± 0.03a	13.98 ± 0.70a
C18:0	5.20 ± 0.03a	4.112 ± 0.80b
C18:1	26.78 ± 0.03a	23.25 ± 1.22b
C18:2	31.85 ± 0.03b	54.74 ± 1.58a
C18:3	1.80 ± 0.01b	5.41 ± 0.80a
CLA *cis,trans/trans,cis*	15.76 ± 0.41	-
CLA *trans,trans*	5.07 ± 0.41	-
Total CLA	20.83 ± 0.00	-
Total saturated fat	18.74 ± 0.04a	18.09 ± 1.07a

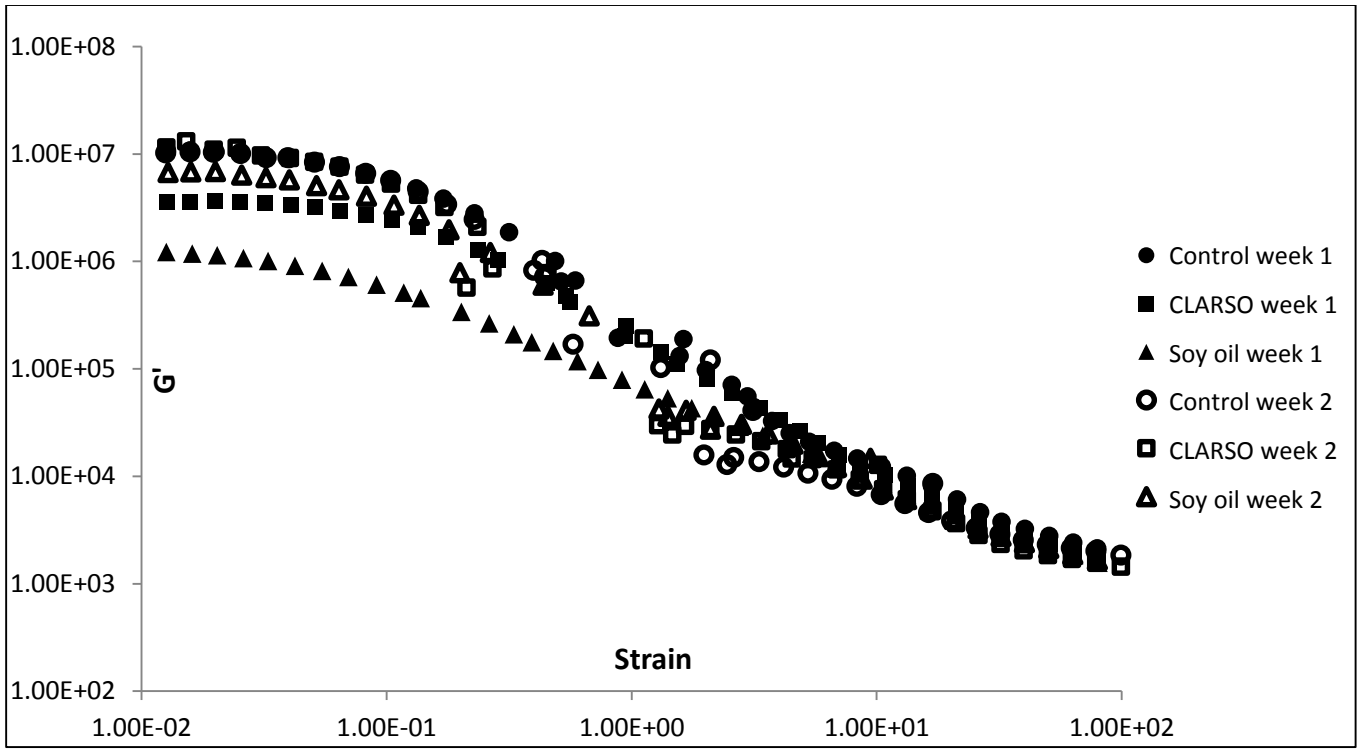

Figure 2.1. Elastic modulus (G') plotted as a function of percent strain for CLA-rich, soy oil, and control chocolate paste samples. Measurements were taken after one and two weeks of storage at 20°C. The analysis was performed in duplicate but the results of only one analysis are shown as the replicates were identical.

Table 2.7- Overall mean G' values for one decade of strain (0.01-0.1) from the strain sweep analysis of CLA-rich, soy oil, and control chocolate paste samples. Analysis was performed in duplicate and error indicates standard deviation. The means were transformed logarithmically to obtain a better comparison. Statistical analysis was performed among all samples. Samples with the same connecting letter are not significantly different.

Sample	Overall mean G'
Control week 1	15.98 ± 0.22 a
CLARSO week 1	14.98 ± 0.14 c
Soy oil week 1	13.69 ± 0.30 d
Control week 2	15.97 ± 0.21 a
CLARSO week 2	16.02 ± 0.28 a
Soy oil week 2	15.51 ± 0.25 b

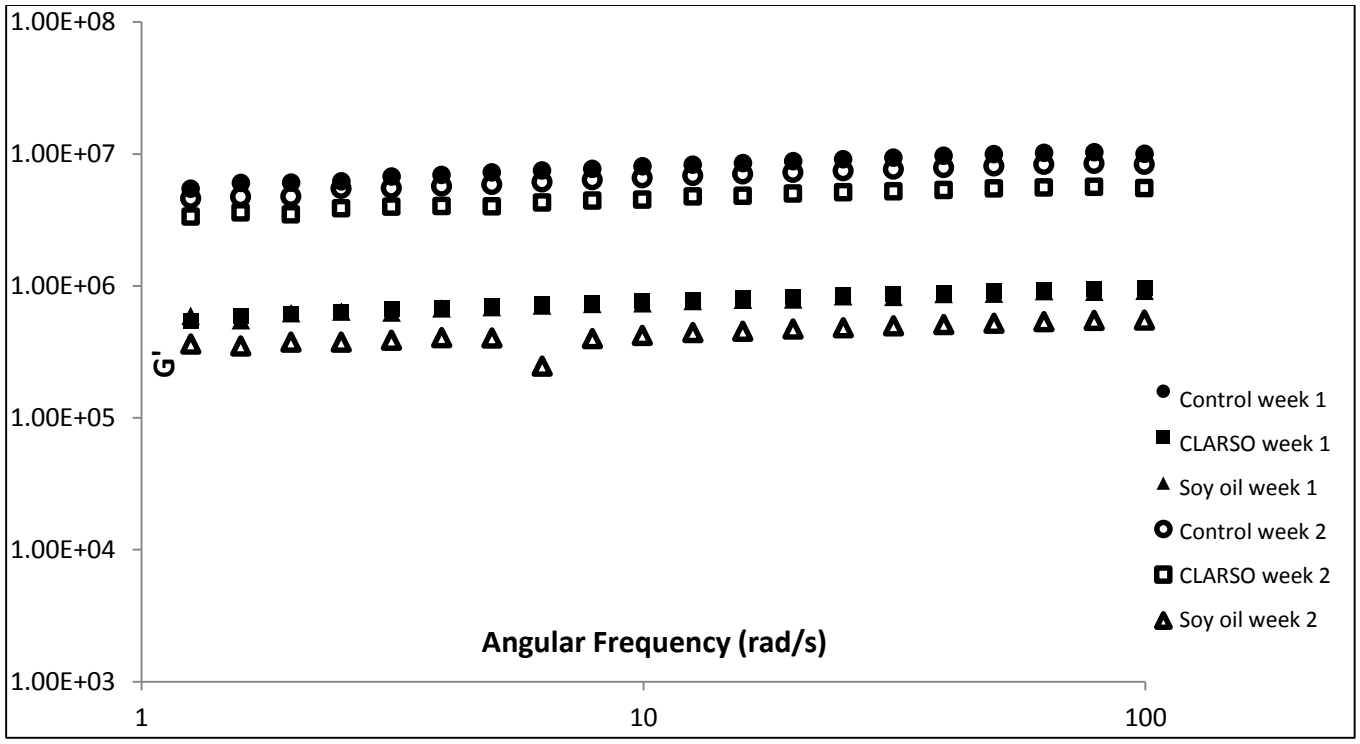

Figure 2.2. Elastic modulus (G') plotted as a function of angular frequency for CLA-rich, soy oil, and control chocolate paste samples. Measurements were taken after one and two weeks of storage at 20°C. The analysis was performed in duplicate but the results of only one analysis are shown as the replicates were identical.

Table 2.8- Overall mean G' values from the frequency sweep analysis of CLA-rich, soy oil, and control chocolate paste samples. Analysis was performed in duplicate and error indicates standard deviation. The means were transformed logarithmically to obtain a better comparison. Statistical analysis was performed among all samples. Samples with the same connecting letter are not significantly different.

Sample	Overall mean angular frequency (rad/s)
Control week 1	15.87 ± 0.22a
CLARSO week 1	13.53 ± 0.17de
Soy oil week 1	13.50 ± 0.18e
Control week 2	15.65 ± 0.27b
CLARSO week 2	15.30 ± 0.21c
Soy oil week 2	12.95 ± 0.21f

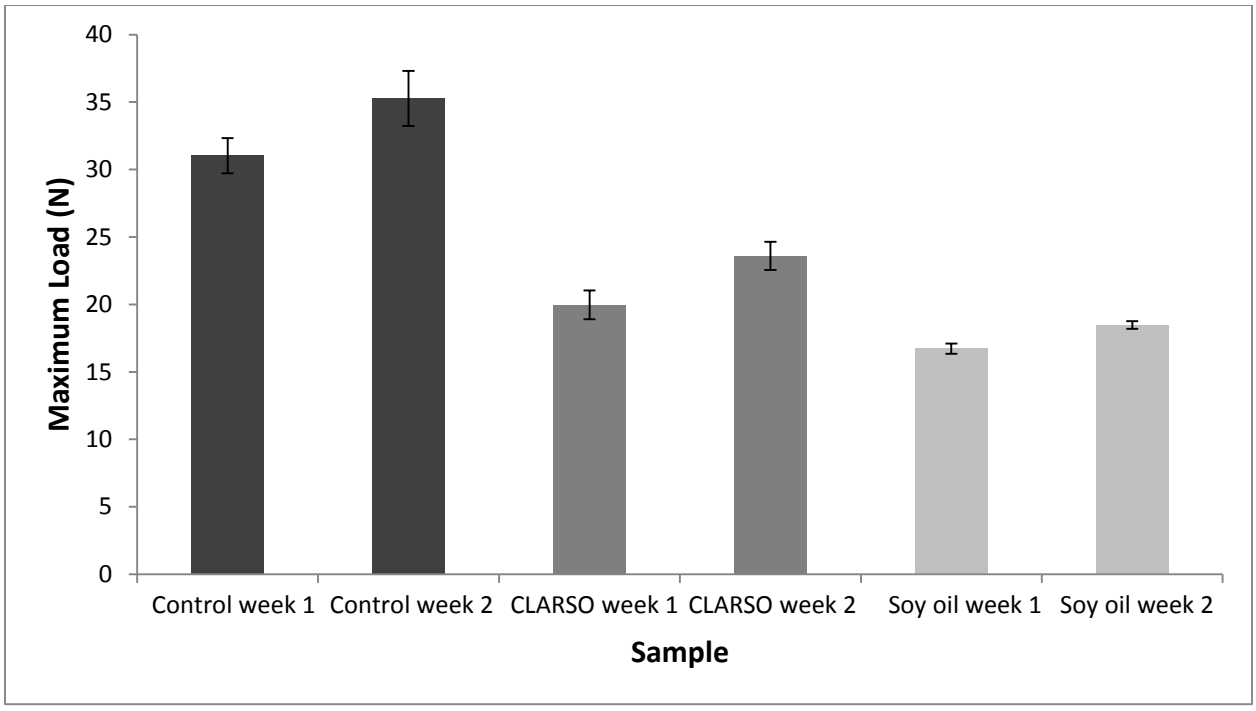

Figure 2.3. Bar graph displaying the mean maximum load measured during firmness analysis of CLA-rich, soy oil, and control chocolate pastes. Measurements were taken after one and two weeks of storage at 20°C. Five sample replicates were analyzed for each type of paste and error bars indicate standard deviation.

Table 2.9- Mean maximum load (N) values from the firmness analysis of CLA-rich, soy oil, and control chocolate paste samples. Analysis was performed on 5 sample replicates and error indicates standard deviation. Statistical analysis was performed among all samples. Samples with the same connecting letter are not significantly different.

Sample	Overall mean maximum load (N)
Control week 1	31.03 ± 1.22b
Control week 2	35.27 ± 1.98a
CLARSO week 1	19.97 ± 1.05d
CLARSO week 2	23.60 ± 1.06c
Soy oil week 1	16.72 ± 0.33f
Soy oil week 2	18.47 ± 0.43e

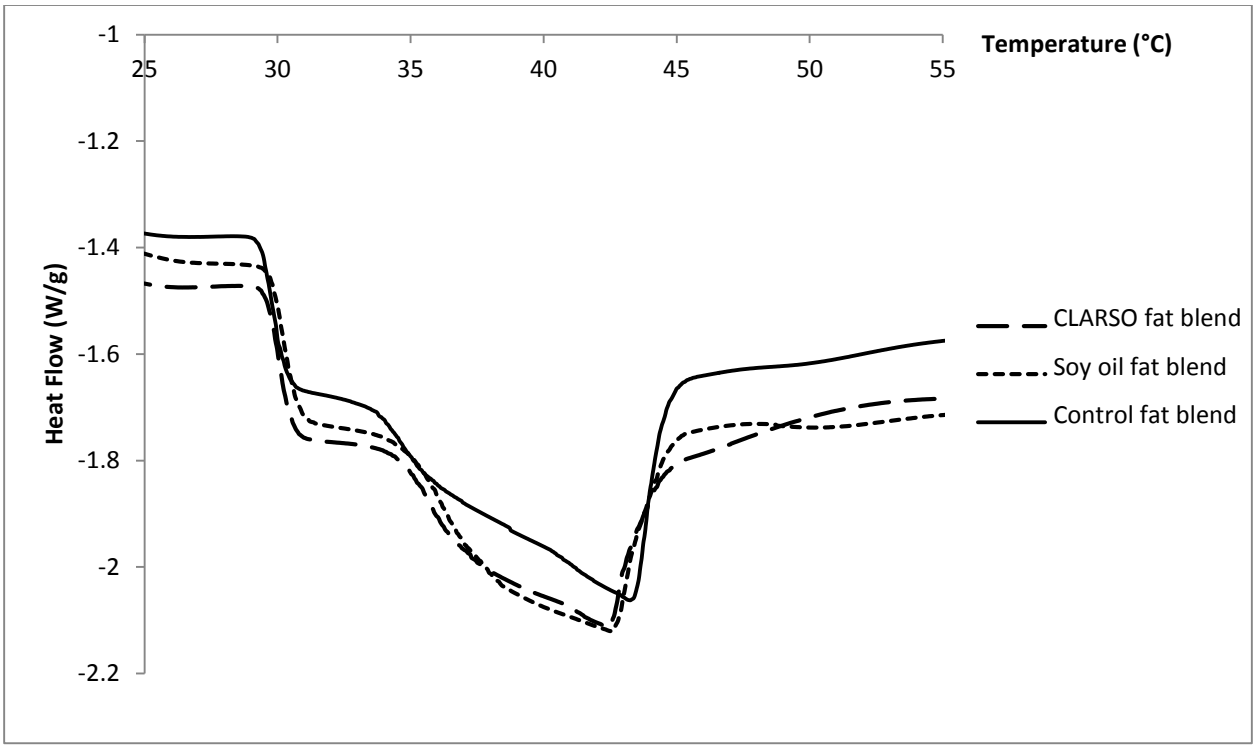

Figure 2.4. DSC spectrum for the melting of CLA-rich, soy oil, and control chocolate pastes, plotted as heat flow (W/g) versus temperature (°C). Analysis was performed in triplicate but only one replicate is shown as the replicates were identical.

Table 2.10- DSC measurements from the melting analysis of CLA-rich, soy oil, and control chocolate pastes. Analysis was performed in triplicate and error indicates standard deviation. Statistical analysis was performed within each column and samples with the same connecting letter are not significantly different.

Sample	Onset Temp. (°C)	Peak Temp. (°C)	Offset Temp. (°C)	Enthalpy (J/g)
CLARSO fat blend	29.46 ± 0.04a	42.36 ± 0.06b	44.87 ± 0.07a	4.04 ± 0.24a
Soy oil fat blend	29.60 ± 0.10a	42.43 ± 0.16b	44.93 ± 0.14a	3.89 ± 0.20a
Control fat blend	29.47 ± 0.13a	43.13 ± 0.23a	44.77 ± 0.35a	4.42 ± 0.28a

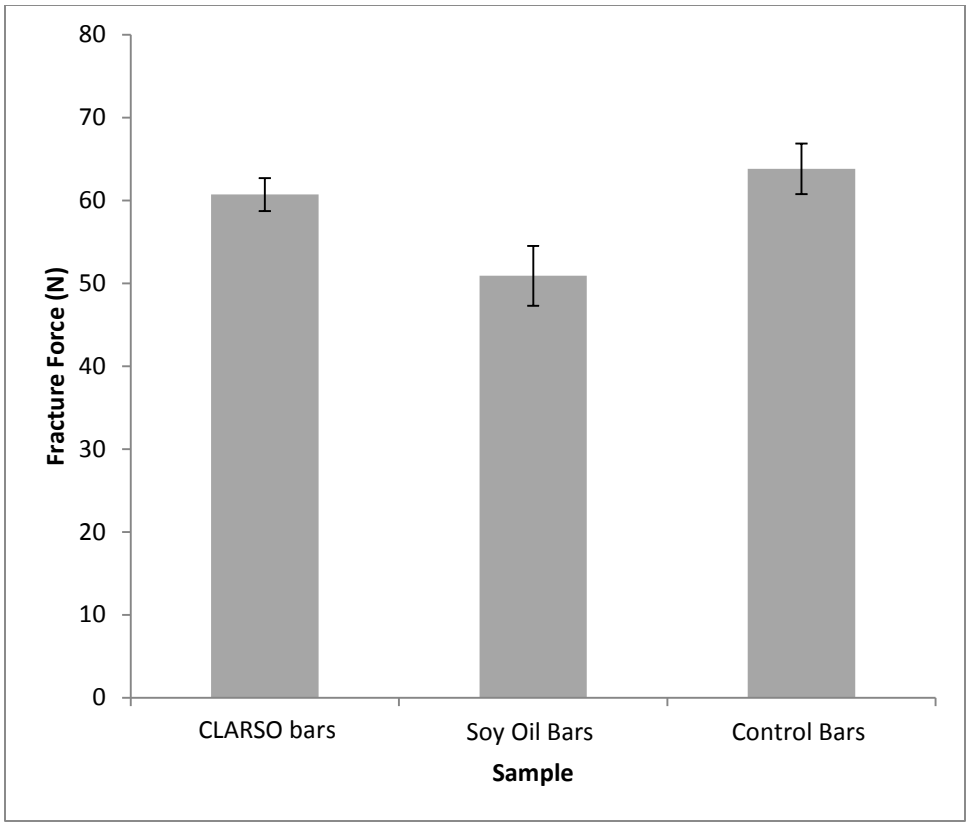

Figure 2.5. Bar graph displaying the mean fracture force of CLA-rich, soy oil, and control chocolate bars. Analysis was performed after one week of storage at 20°C. Ten sample replicates were analyzed for each type of bar and error bars indicate standard deviation.

Table 2.11- Mean fracture force (N) values from the fracturability analysis of CLA-rich, soy oil, and control chocolate bars. Analysis was performed on 10 sample replicates and error indicates standard deviation. Statistical analysis was performed among all samples. Samples with the same connecting letter are not significantly different.

Sample	Fracture force (N)
Control bars	63.80 ± 3.05a
CLARSO bars	60.70 ± 2.00a
Soy oil bars	50.90 ± 3.60b

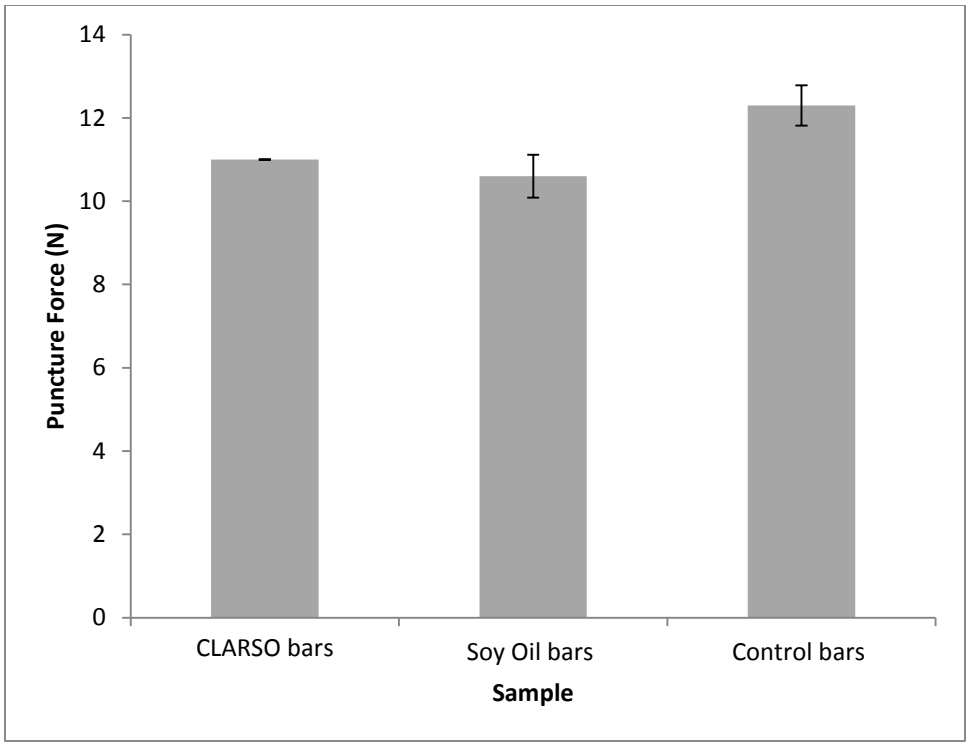

Figure 2.6. Bar graph displaying the mean maximum load measured during hardness analysis of CLA-rich, soy oil, and control chocolate bars. Analysis was performed after one week of storage at 20°C. Ten sample replicates were analyzed for each type of bar and error bars indicate standard deviation.

Table 2.12- Mean puncture force (N) values from the hardness analysis of CLA-rich, soy oil, and control chocolate bars. Analysis was performed on 10 sample replicates and error indicates standard deviation. Statistical analysis as performed among all samples. Samples with the same connecting letter are not significantly different.

Sample	Puncture force (N)
Control bars	12.30 ± 0.48a
CLARSO bars	11.00 ± 0.00b
Soy oil bars	10.60 ± 0.52b

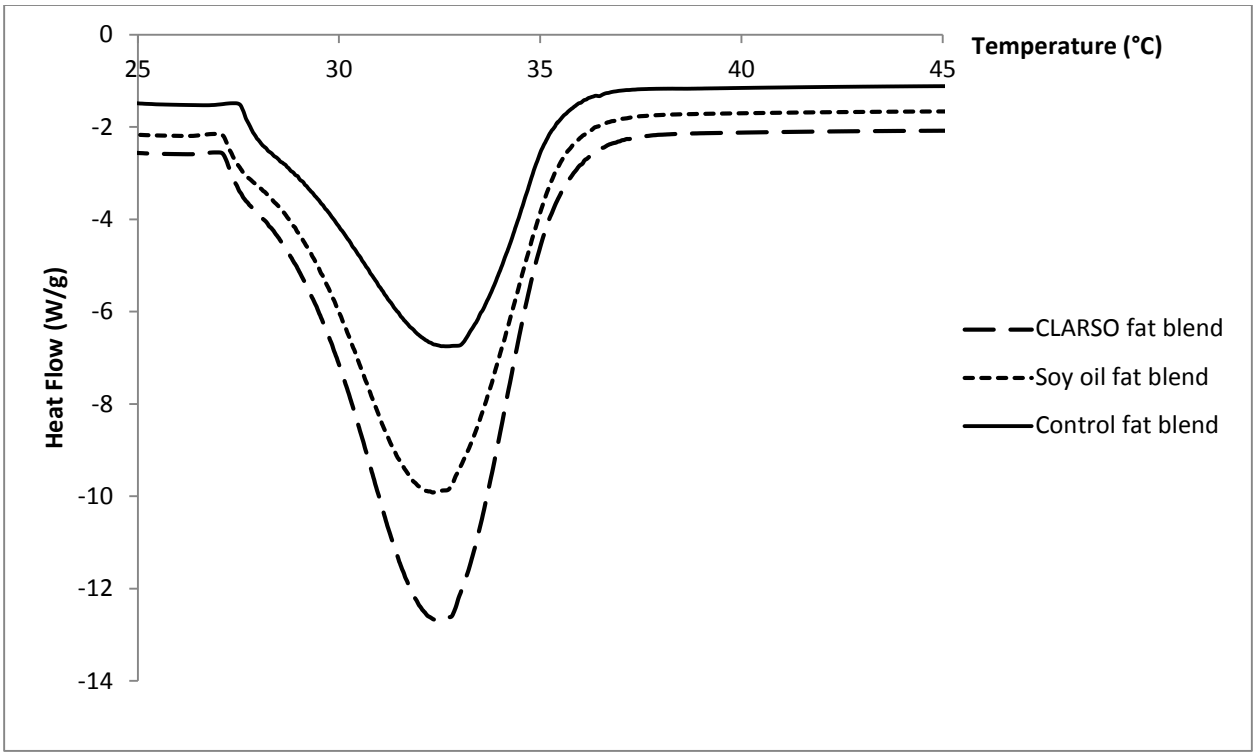

Figure 2.7. DSC spectrum for the melting of CLA-rich, soy oil, and control chocolate bars, plotted as heat flow (W/g) versus temperature (°C). Analysis was performed in triplicate but only one replicate is shown as the replicates were identical.

Table 2.13- DSC measurements from the melting analysis of CLA-rich, soy oil, and control chocolate bars. Analysis was performed in triplicate and error indicates standard deviation. Statistical analysis was performed within each column and samples with the same connecting letter are not significantly different.

Sample	Onset Temp. (°C)	Peak Temp. (°C)	Offset Temp. (°C)	Enthalpy (J/g)
CLARSO Bars	27.20 ± 0.06b	32.75 ± 0.32a	37.54 ± 0.64a	37.62 ± 0.89a
Soy Oil Bars	27.23 ± 0.08b	32.55 ± 0.20a	36.85 ± 0.21a	35.85 ± 1.68ab
Control Bars	27.51 ± 0.08a	32.29 ± 0.32a	36.33 ± 0.61a	32.72 ± 1.09b